THE AMERICAN JOURNAL OF PATHOLOGY

THE AMERICAN JOURNAL OF PATHOLOGY

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THE AMERICAN JOURNAL OF PATHOLOGY

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THE INFECTIVITY OF ISOLATED INCLUSION BODIES OF FOWL-POX *

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The group of so-called filterable virus diseases is coming to occupy an increasingly important place in medical and biological literature. Included in this group is fowl-pox or epithelioma contagiosum of birds.[1] In common with many of the other virus diseases fowl-pox is characterized by the presence of inclusion bodies in the affected epithelial cells. Ever since their discovery [2] the question has been raised with regard to these bodies as to whether they represent a mere degeneration product of the cell,[3, 4] or whether, in some fashion, they actually carry the infectious agent of the disease.[5, 6]

In recent publications from this laboratory [7, 8] morphological evidence has been presented which seems to justify the opinion that the inclusion bodies represent colonies of a minute microörganism (bodies of Borrel [9]) enveloped in a membrane of lipoproteid composition which is semipermeable. The inclusions, when placed in distilled water, swell, with the formation of vacuoles in which numbers of rounded uniform bodies measuring about 0.25 micron in diameter can be seen in rapid Brownian motion. The addition of sodium chloride causes the bodies to shrink and to assume again their initial hyaline, homogeneous appearance. A crushed inclu-

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sion, suitably stained, discloses the minute bodies enclosed in a faintly staining homogeneous material which is regarded as a sort of brood capsular material for a living agent represented by the minute granules. It was in the attempt to shed some added light on the question of infectivity of the inclusion bodies that the following series of experiments was undertaken.

The characteristic lesion of spontaneous fowl-pox is a local epithelial hyperplasia. The virus proliferates locally in these eruptive cutaneous lesions and material from them is highly infectious. So far as determined an increase in virus is limited to these areas of epithelial hyperplasia. Only squamous epithelium of the skin and mucous membranes seems to be susceptible to the virus. Experimental infection is readily induced by applying active virus to any superficially injured squamous epithelial surface. If a feather be plucked from the breast of a hen and the follicle inoculated with material from an eruptive lesion a slight swelling of the follicle may be noted after three days. After seven to ten days the follicle has increased to about ten times its original size. This is shown in Fig. 1 in which the follicle "*a*" had been inoculated ten days previously. The other follicles remain normal. By carefully dissecting away the skin the swollen epithelial core of the follicle may be shelled out as shown in Fig. 2. This particular core is about 1½ mm. in diameter and about 3 mm. long. If a section be made of it the core is found to consist of concentric layers of swollen epithelial cells in each one of which may be seen one or more of the inclusion bodies (Fig. 3). The swelling of the follicle is due both to an hyperplasia of the epithelium and to a swelling of the individual cells in the stratum mucosum. The cells in this region are enlarged to several times the size of those in the stratum germinativum. The eosin-staining inclusion body is usually in the center of the cell, with a broad, non-staining zone between the inclusion and the cell membrane. The bodies may also be distinctly seen within the epithelial cells in a fresh preparation of the diseased tissue teased out in saline.

The question regarding the nature of the inclusion bodies in fowl-pox has been approached from a new angle through the discovery that, using a 1 per cent solution of trypsin in 0.2 per cent sodium bicarbonate, the cellular material of a fowl-pox lesion may be digested away completely leaving the inclusion bodies free. This process of freeing the inclusion bodies by digestion is a striking

thing to watch. If the core of epithelium just illustrated be cut into small pieces and placed on a glass slide along with a few drops of trypsin the inclusion bodies begin to show more distinctly almost immediately and, after five minutes in the solution, many of the bodies drop free from the tissue to lie like minute pearls on the surface of the slide (Fig. 4). After thirty minutes almost all of the inclusion bodies are free as shown in Fig. 5, and the amorphous mass of digested epithelium and connective tissue may be shaken about with a needle and separated completely from any remaining inclusion bodies.

The way in which the epithelial cells of a fowl-pox lesion are digested away completely, leaving the inclusion bodies unchanged, in itself suggests that these bodies represent something more than a degeneration product of the cell. As shown in Fig. 6, taken forty-five minutes after the beginning of digestion, the inclusion bodies remain intact while all that remains of the epithelium is the amorphous material seen as the groundwork of the picture.

The material which may protect the inclusion bodies from tryptic digestion is a fatty element readily demonstrable by means of special fat stains. In addition to the fat there is an albuminous component of the body.[10] The structures are elastic and may be indented with a needle only to regain their shape as soon as the needle is removed. In general the bodies are rounded or oval as shown in Fig. 7. Occasionally one may be bean-shaped or otherwise irregular. The irregularity in shape occurs usually in the larger bodies, the smaller ones being almost always rounded. In size the bodies vary from 2 or 3 microns to perhaps 50 microns in diameter. They are of relatively high density and quickly gravitate to the bottom if suspended in a physiological saline solution. They may be readily rolled about in saline on a glass slide though an occasional body may adhere to the slide and leave a small portion of its substance if broken away. The bodies are highly refractive. As shown in Fig. 7 they look, under the higher magnification of the microscope, like miniature white potatoes.

The compact nature of the inclusion bodies when freed by digestion makes them very easily manipulable for the purpose of inoculation. The trypsin may be pipetted from the surface of the bodies and be replaced with saline. This in turn may be pipetted off and the process repeated until the bodies appear to be free from débris.

After each addition of saline the bodies may be agitated in the solution since they rapidly settle to the bottom again. Those bodies which happen to strike the surface of the solution form an exception to the rule for, once on the surface, they are closely held there by surface tension. After some experimenting it was found that a 2 per cent salt solution seemed to keep the bodies slightly more firm and dense than physiological saline, so the 2 per cent solution was used exclusively for the fluid medium in our experiments.

After freeing the inclusion bodies from all cellular material an attempt was made to test their infectivity. Former attempts in this direction have been hampered by the failure to get the bodies cleaned sufficiently from their cellular surroundings. The process of digestion encouraged us in the belief that the inclusion bodies could be freed and washed clean enough to make their inoculation of value in determining whether or not the inclusion carries the infective agent of the disease. Among all of the inclusion body diseases, fowl-pox lends itself with particular readiness to this experiment because of the large size and the compactness of the inclusions. Furthermore a bird is inoculated with extreme ease since the simple operation of plucking a feather leaves an epithelium-lined follicle which is an ideal nidus for the inoculation of minute amounts of fluid.

For picking up the individual inclusion bodies a Chambers micro-dissection apparatus was found necessary. Two variations from the usual Chambers technic for isolating single bacteria by the pipette method were employed. Instead of using a hanging drop it was necessary to work with a small pool of material on the upper surface of the slide. This was because the inclusion bodies in the hanging drop fell to the dependent surface of the fluid, and once they had come under the influence of surface tension it was found impossible to remove them from the fluid. The second change found necessary was the introduction of a micro-adjustment for the plunger of the injection syringe. This syringe, when worked with the unaided hand, forced a veritable torrent of fluid through the opening of the fine capillary pipette to which it was attached. With a simple screw attachment for the plunger it was found possible to regulate perfectly the flow of fluid through the capillary opening.

The apparatus is used under the low power of the microscope with the light cut down so that the inclusion bodies show up promin-

ently on a dim field. The digesting and washing are done with the help of a dissecting microscope.

In the first inoculation experiment a small piece of infected epithelium was digested in trypsin for one and a half hours. The inclusion bodies were then washed with saline. About a hundred, as seen under the dissecting microscope, were sucked into a large pipette, transferred to another slide, and this slide was introduced under the microscope with the Chambers attachment. From five to ten inclusion bodies were sucked into a fine capillary pipette and inoculated into a breast follicle from which the feather had been plucked. Since the object of our experiment was to find whether the virus is concentrated in the inclusion or in the cellular material about it, the inoculation of inclusion bodies was controlled by the inoculation of the fluid overlying them into a follicle on the opposite side of the chicken. This fluid was by no means clear but contained numerous small particles floating in it. Consequently, when after seven days a bilateral "take" occurred on the chicken, it was thought that possibly a small inclusion body or portion of inclusion had been contained in the supernatant fluid of the control inoculation.

A refinement of technic was next attempted by picking up five or ten inclusion bodies in the capillary pipette with a minimum of fluid, transferring these to an entirely new pool of saline and from this pool picking up the inclusion bodies with a fresh pipette for inoculation into the fowl. However, this experiment too showed a "take" for both the inclusion body inoculation and for the supernatant fluid.

In the hope that a further washing of the inclusion bodies might give a supernatant fluid free from virus the bodies were next passed through two distinct pools of saline. A sample protocol of the digesting and washing process employed may be roughly diagrammed as follows, using Hen P as an illustration.

1 { Digesting pool under dissecting scope } Several hundred inclusion bodies transferred to

2 { Sorting pool under Chambers scope } 21 inclusion bodies picked up 14 delivered to

3 { First wash pool } 7 inclusion bodies picked up 4 delivered to

4 { Second wash pool } 4 inclusion bodies inoculated into anterior breast follicle, right side of Hen P.
Supernatant fluid inoculated into anterior breast follicle, left side of Hen P.

The amount of fluid sucked into the capillary pipette in the transfer from Pool 2 to Pool 3 and again from Pool 3 to Pool 4 was in each instance not over .01 cc. Pools 3 and 4 each consisted of at least 1 cc. of saline, so that the fluid carried over from Pool 2 was diluted 1 to 100 in the first transfer and again 1 to 100 in the transfer from Pool 3 to Pool 4, making a final dilution of at least 1 to 10,000 of any fluid carried over from Pool 2.

In three different experiments chickens were inoculated on the right breast with inclusion bodies washed in this fashion and on the left breast with supernatant fluid equal in amount to the fluid inoculated with the bodies. The results of these experiments were uniformly positive on the side inoculated with inclusion bodies and negative on the side inoculated with their supernatant fluid. The diagnosis of a positive result was based on a demonstration of inclusion bodies in the lesion and on its content of active virus as determined by inoculation.

Chart Showing Results of Inoculations with Washed Inclusion Bodies

Follicle Number	Number of Bodies Inoculated	Result	Control
N_1	6	+	−
O_1	2	+	−
P_1	4	+	−
K_1	1	−	−
M_1	1	+	*
T_1	1	−	−
T_2	1	+	−
U_1	1	−	−
U_2	1	−	−
U_3	1	−	−
V_1	1	−	−
V_2	1	−	−
V_3	1	−	−
W_1	1	+	−
W_2	1	+	−
W_3	1	−	−
WH_1	1	+	−
WH_2	1	+	−
WH_3	1	+	−

* No control

The question next arose as to how many inclusion bodies must be inoculated in order to secure a "take." In the preceding experi-

ments the average number of inclusion bodies inoculated into each follicle had been four. A series of sixteen follicles was next inoculated with one inclusion body in each follicle. As shown in the chart seven of these inoculations showed a "take" while nine were negative. In no instance, however, was a single control inoculation positive.

A probable explanation of the failure to secure positive results in a larger percentage of the single inclusion body inoculations seemed to lie in the difficulty experienced in delivering a single body from the pipette into the follicle. As graphically illustrated for Hen P only fourteen of the twenty-one inclusion bodies picked up could be delivered into the first wash pool and only four out of seven could be delivered into the second wash pool. This example is characteristic of our experience in all of the attempts to transfer the inclusion bodies from one pool to another and indicates a propensity of the bodies for sticking to the wall of the pipette. Considering this difficulty the low percentage of "takes" is not remarkable. There was no gradation in the size or in the time of appearance of the "takes," those which appeared at all coming on just as quickly as those which had been inoculated with four or five inclusion bodies.

The lesion caused by the inoculation of one inclusion body is shown in Fig. 8. Each of the two swollen follicles was inoculated seven days previously with a single inclusion body. The swollen follicles are 2 to 3 mm. in length and contain several hundred thousand inclusion bodies similar to the single inclusion body with which each was originally inoculated. The anterior one of these two follicles was removed and digested so as to free the bodies. The digestion mixture was then smeared on the plucked and scarified breast of a chicken. The lesion resulting after seven days is shown in Fig. 9. The massive "take" in the plucked follicles and along the lines of scarification is, then, the result in two weeks time of the inoculation of a single inclusion body.

Discussion

The rapid development of a lesion following inoculation with a single inclusion body we have interpreted as being due to the inoculation not of a single microörganism but of a large number enclosed within the inclusion. Special stains of crushed inclusions show by

count hundreds of definite, rounded objects about 0.25 micron in diameter within each body.[7] These minute objects are believed to represent the actual virus of fowl-pox. Consequently, in inoculating a single inclusion body, one is probably inoculating an entire colony of virus bound up in its fatty capsule.

Working with the digested material from a fowl-pox lesion gives one a very definite impression as to the character of the inclusion bodies. The way in which the bodies resist tryptic digestion marks them as something more substantial than the cell which contains them. The way in which the bodies can be washed clean and inoculated to give a "take," while their supernatant fluid remains sterile, indicates that the inclusion bodies actually carry the infectious agent of the disease. The way in which a single inclusion body, when inoculated, produces in two weeks time a lesion containing millions of similar bodies indicates that the inclusion bodies of fowl-pox contain and are caused by a living, growing virus. So certain have we become that the inclusions carry a virus that the bodies are now spoken of with confidence as *virus* bodies rather than inclusion bodies.

The results of these experiments afford additional evidence that the typical lesion of fowl-pox is induced through an invasion of epithelial cells by the virus and its intracellular proliferation locally within the lesion.

Summary

1. The inclusion bodies of fowl-pox are composed of hundreds of minute bodies enclosed in a fatty capsule.

2. The hyperplastic epithelium of the lesion of fowl-pox, when subjected to tryptic digestion, liberates the intact inclusion bodies while the epithelial cells undergo complete digestion.

3. A single inclusion body, when washed with saline and inoculated into the skin of the hen, has produced a typical fowl-pox lesion containing the characteristic inclusions. The fluid in which the inclusion body is finally suspended is innocuous.

4. The inclusion bodies of fowl-pox are interpreted as being true *virus* bodies, *i.e.*, minute colonies of the etiological agent of the disease.

5. The proliferation of the virus is accordingly largely, if not entirely, intracellular.

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DESCRIPTION OF PLATES

PLATE 1

FIG. 1. Swollen feather follicle at "*a*," ten days after inoculation with a single inclusion body of fowl-pox.

FIG. 2. Epithelial core of inoculated feather follicle. This particular core was removed from the anterior follicle illustrated in Fig. 8.

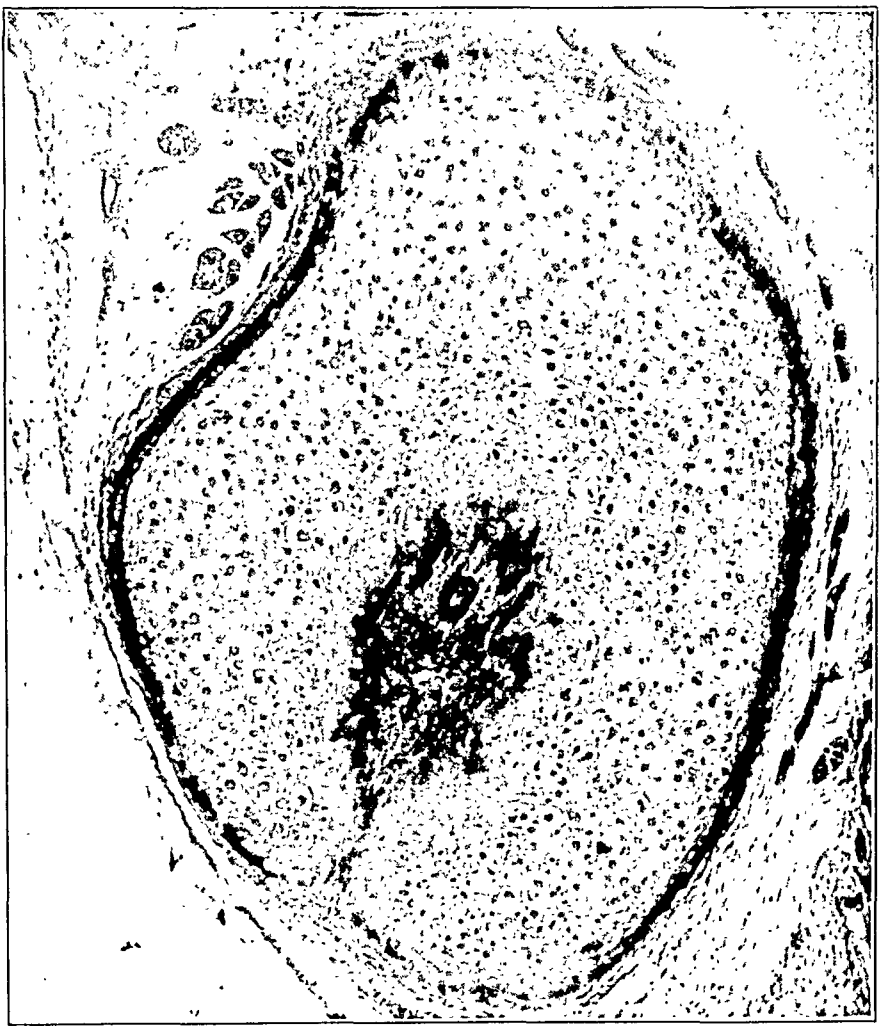
FIG. 3. Section through feather follicle removed ten days after inoculation with fowl-pox. The dark bodies within the swollen epithelial cells are the inclusion bodies. Hematoxylin and eosin. × 80.



1



2

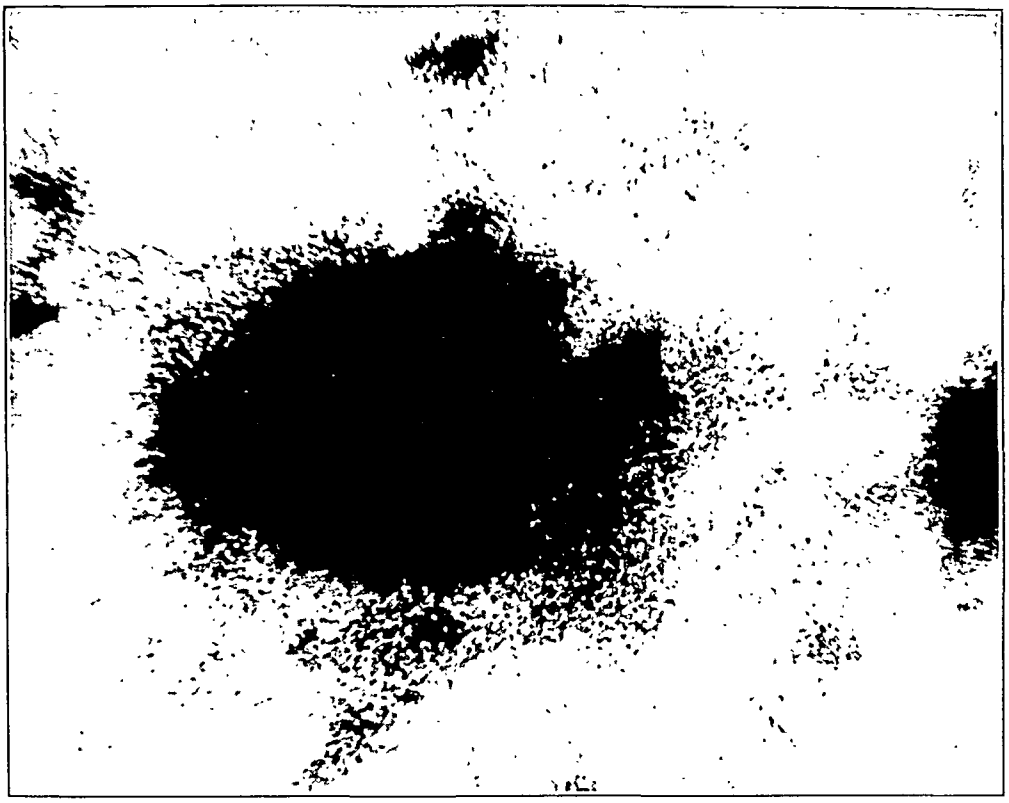


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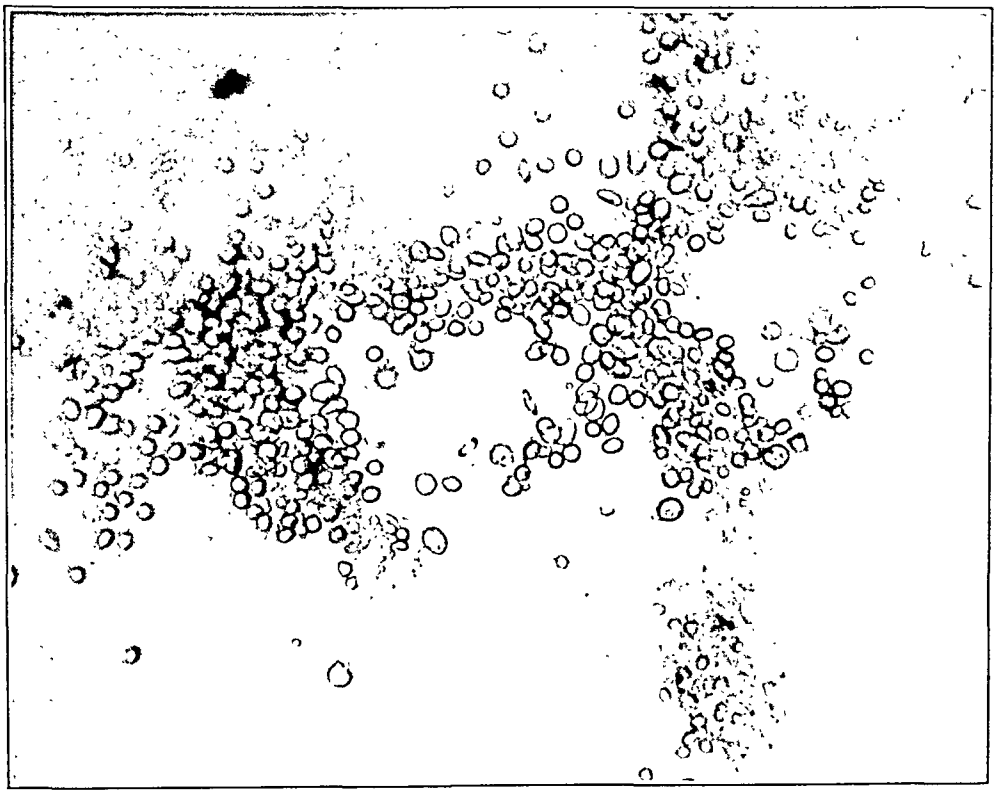
PLATE 2

FIG. 4. Mass of infected epithelial cells in the process of digestion. Some of the freed inclusion bodies may be seen on the right-hand edge of the epithelial mass. × 47.

FIG. 5. Inclusion bodies thirty minutes after the beginning of digestion. × 160.



4

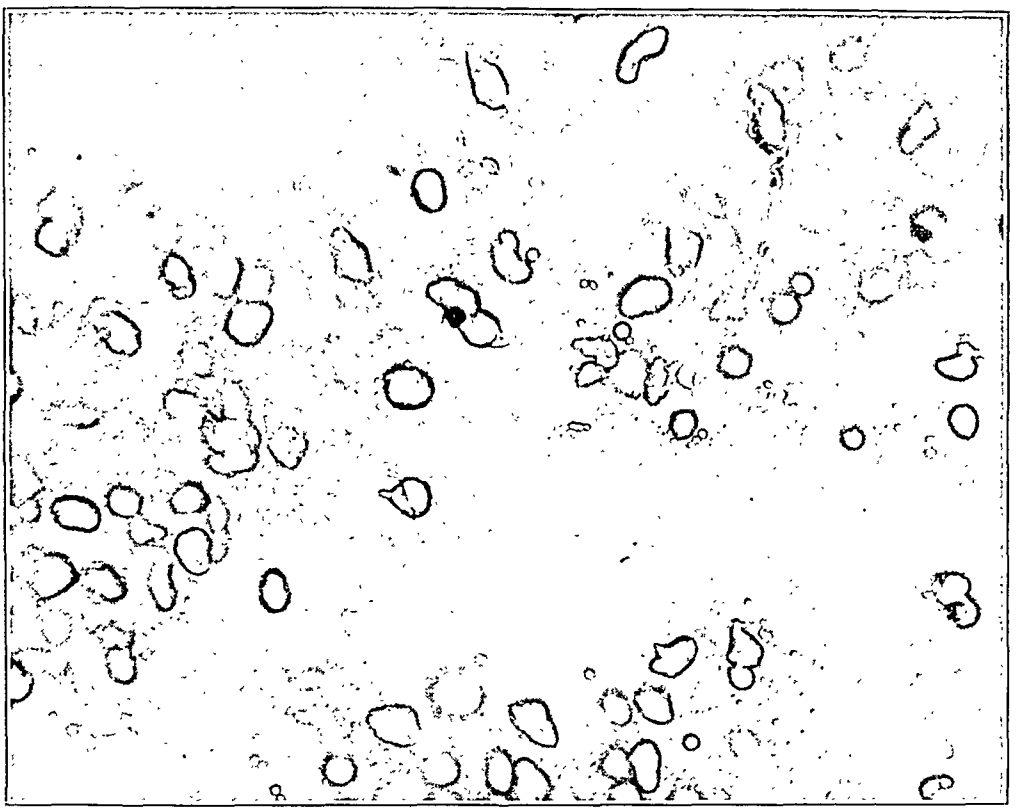


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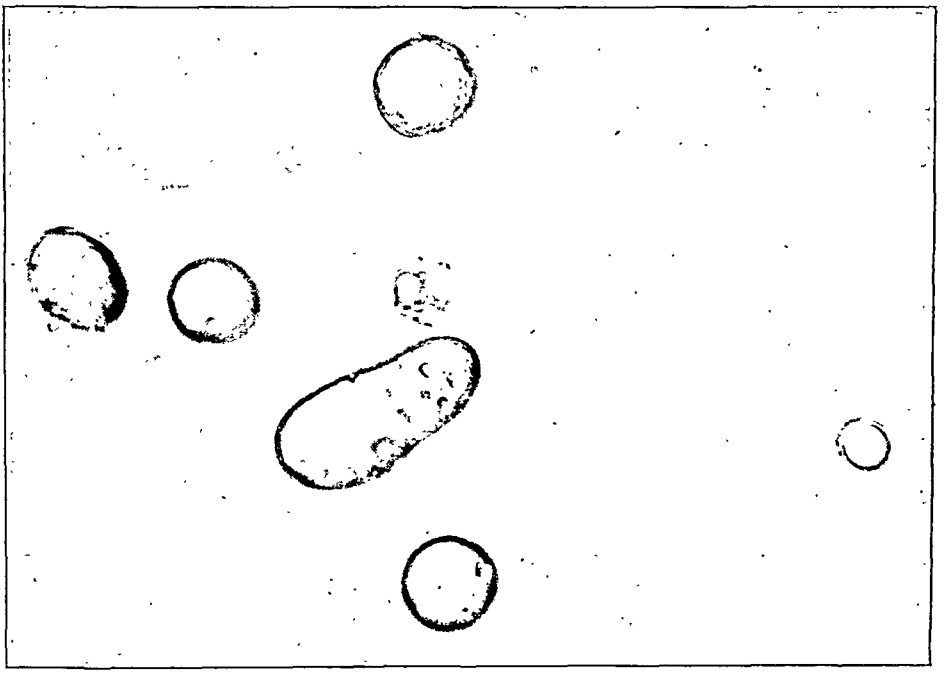
PLATE 3

FIG. 6. Inclusion bodies forty-five minutes after the beginning of digestion. Amorphous material forming the groundwork of the picture represents the digested epithelium. × 330.

FIG. 7. High magnification of inclusion bodies completely freed by digestion. × 1000.



6

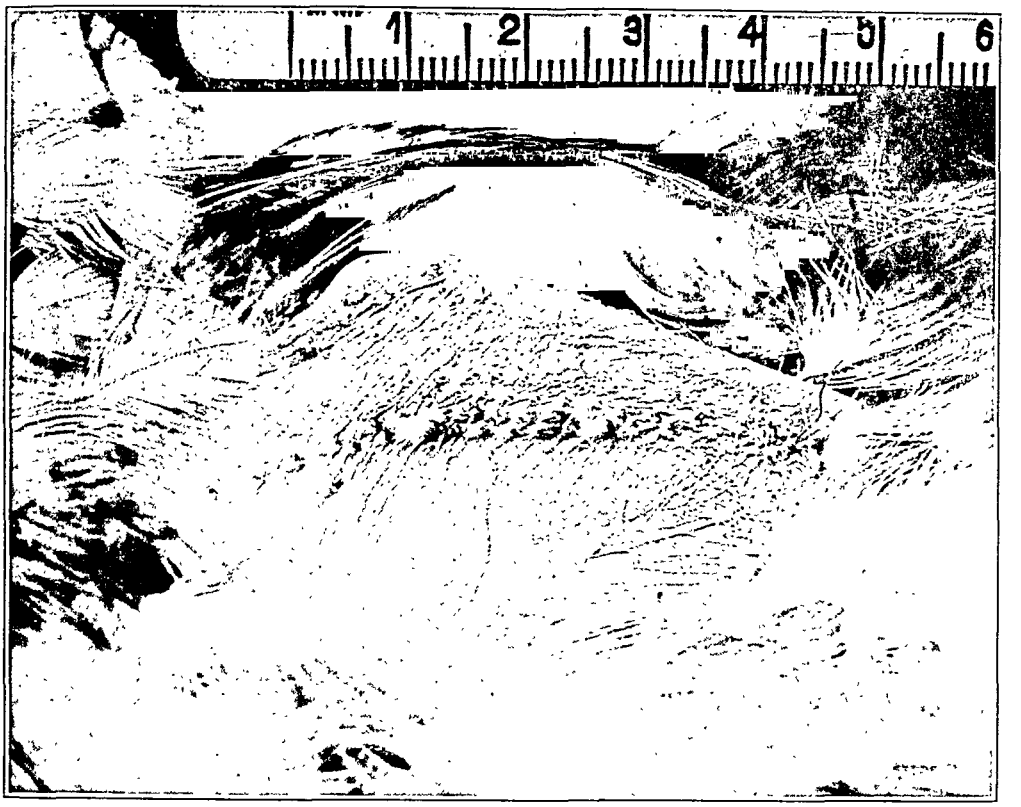


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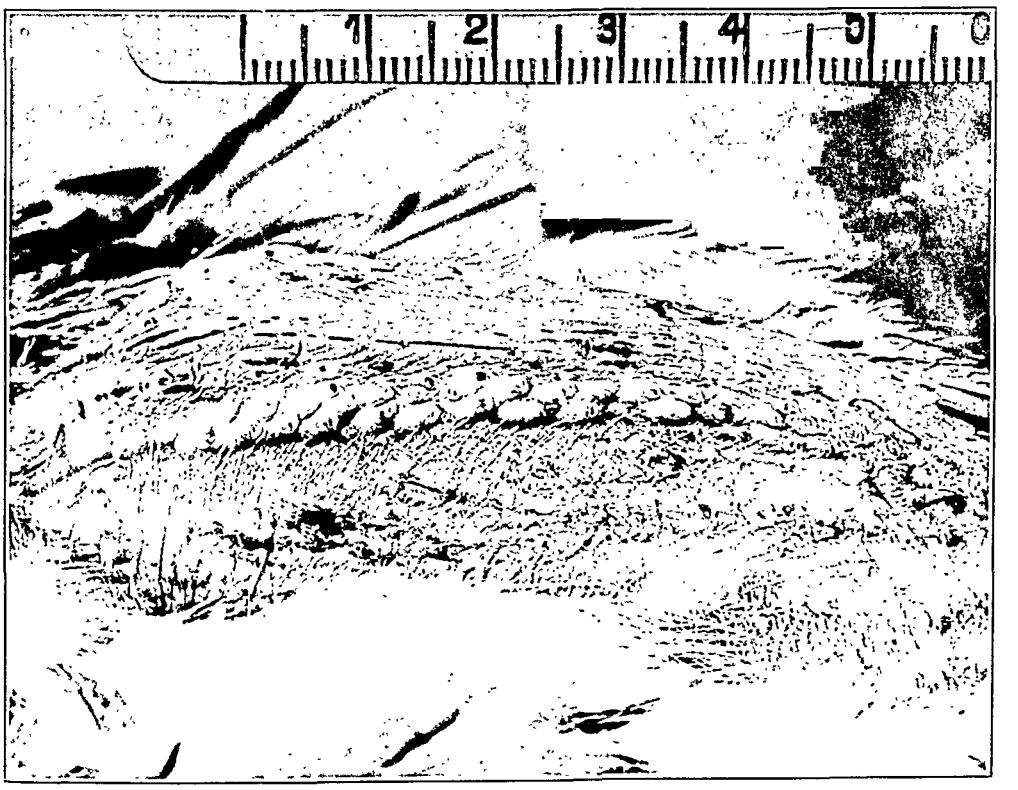
PLATE 4

FIG. 8. Two feather follicles each of which had been inoculated seven days previously with a single inclusion body.

FIG. 9. Plucked and scarified breast of hen seven days after inoculation with inclusion bodies from the anterior follicle illustrated in Fig. 8.



8



9

A CASE OF EXTENSIVE BILATERAL PROGRESSIVE THROMBOSIS OF THE SMALLER BRANCHES OF THE PULMONARY ARTERIES *

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In order to emphasize the importance of disease in the pulmonary arteries Posselt [1] in 1909 published an extensive and comprehensive review of the lesions in these vessels that had been reported over a long period of years. He divided the lesions in the pulmonary arteries into groups depending upon the nature of the lesion, whether primary or secondary, whether associated with acquired or congenital cardiac disease and whether there was disease in the other arteries of the body. Among the various lesions described Posselt refers to eighteen reports of cases of thrombosis with endarteritis of the pulmonary arteries. In some of these cases the thrombosis was extensive and occurred sometimes in the smaller branches of the pulmonary arteries but the distribution of the lesions and the clinical picture of none were sufficiently similar to the case here reported to identify any of them as the same disease. For a very complete review of the literature up to 1909 one is referred to this article.

The causes assigned to the production of the various vascular lesions in the pulmonary arteries described in Posselt's review were often somewhat obscure although special disorders seemed to be associated with certain of the vascular changes. Thus conditions which caused an increase of pressure in the pulmonary circulation such as passive congestion from left-sided cardiac disease, emphysema, disease of the pleura and pulmonary tuberculosis were blamed for lesions in the pulmonary arteries. In like manner syphilis, the acute infections, especially smallpox, and congenital weakness of the blood vessels were considered as etiological factors. Kitamura [2] thought that plethora from excessive beer drinking was a cause of lesions in the pulmonary arteries and Emery [3] the misuse of alcohol. For the cases with thrombosis in the pulmonary arteries no special etiological factor was mentioned as a probable cause.

* Received for publication September 7, 1928.

Most of these cases of disease of the pulmonary arteries showed hypertrophy of the right side of the heart, in some instances even very extensive hypertrophy. Most of them also showed cardiac failure with edema or dropsy in some parts of the body.

Since that very complete review by Posselt in 1909 there have appeared in the literature a few reports of disease of the pulmonary arteries but on the whole the subject does not seem to have received much attention. Most of the articles have laid emphasis on the disturbance in the heart that pulmonary vascular lesions may cause. Sanders [4] in 1909 reported a case of sclerosis of the smaller branches of the pulmonary artery without offering any reason for the lesions. Arrillaga [5] in 1913 published a monograph on the clinical syndrome of chronic cyanosis, dyspnea and erythremia described by Ayerza in 1901 as due to sclerosis of the pulmonary artery which has been spoken of as Ayerza's disease. These lesions were in the larger branches and at the origin of the pulmonary artery. The cause of this sclerosis was not made clear. Warthin [6] in 1919 ascribes this arterial lesion to syphilis and there is apparently no doubt that syphilis does produce changes in this artery. Moschcowitz [7] in 1927 claimed that the lesions of Ayerza's disease were not due to syphilis but were due to increased pressure in the lesser or pulmonary circulation.

In 1915 Veale and Coombs [8] described gross sclerosis in the pulmonary arteries which they spoke of as primary because no apparent cause was found. In 1914 Schütte [9] reported a case in which the lesions in the pulmonary arteries were in the smaller branches and were detected only under the microscope. He offered no cause for the lesions but the patient was 74 years old and the lesions might have been the result of age, although the pictures suggest that they might have been the result of organization of thrombi. Also in 1916 Hart [10] reported two cases of sclerosis of the pulmonary arteries without apparent cause.

Krutzsch [11] in 1920 reported on the lesions that may occur in the pulmonary arteries and of special interest was his report of a case with thrombi in the small arteries and capillaries of the lungs associated with a wide dissemination of cancer cells. Gamna [12] in 1921 pointed out that sclerosis of the pulmonary arteries occurred in congenital cardiovascular malformations and chronic pleuropulmonary disorders. The sclerosis in these conditions usually involved the

larger branches. He also claimed that a primary sclerosis occurred in the smaller branches of the pulmonary arteries without any apparent cause. The lesions often simulated endarteritis obliterans. Eppinger and Wagner [13] in 1920 called attention to lesions in the smaller branches of the pulmonary arteries without apparent lesions in the larger branches so that nothing was suspected on gross examination. In their cases there were often thrombi in the small arteries, and they resembled in many ways the one here reported. They suggested that underlying the thrombi in the arteries there were demonstrable lesions of the arterial walls. They discussed the various causes of vascular lesions without reaching any definite conclusions and pointed out that many of the theories regarding the cause of these vascular lesions did not fit their cases. In 1927 Clark, Coombs, Hudfield and Todd [14] reported five cases of disease in the pulmonary arteries in one of which there was an obliterating endarteritis which they thought due to syphilis and one case with thickening and nodules in the branches of the pulmonary arterial tree that were only from five to one millimeters in diameter. The cause for the lesions in these small arteries was not apparent.

It is quite obvious, therefore, that various lesions occur in the pulmonary arteries and that these lesions may occur in the larger or smaller branches alone, or in both. Syphilis undoubtedly causes some of the lesions. Other lesions similar to arteriosclerosis also occur without the etiology being any more apparent than it is in arteriosclerosis elsewhere in the body. In this group increase in the intra-pulmonary pressure, diseases of the lungs or pleura and acute infections are among the causes blamed for the arterial lesions. Congenital defects are also considered as a cause of some of the vascular lesions. In addition a few instances of thrombotic lesions with obscure etiology in the smaller pulmonary arteries have been reported.

The following case, in which interesting lesions were found in the pulmonary arteries, was observed clinically and came to autopsy.

Case Report

Clinical Record: Peter Bent Brigham Hospital Number 51761.

The patient was a single, white woman, aged 38 years who entered the Peter Bent Brigham Hospital on April 15, 1926, and died on April 24, 1926. Her chief complaint was shortness of breath.

The family history was unimportant. In childhood she was ill with chickenpox, measles, mumps, diphtheria, whooping cough and possibly typhoid fever.

All her life she had been subject to frequent "colds" and had been somewhat deaf for twenty years. Five years before entry her tonsils were removed.

The exact time of the onset of her final illness was not clear, but she claimed that she was perfectly well up to about six months before admission to the Brigham Hospital. At that time while having a severe "cold" in the head she noticed marked shortness of breath while on a mountain climbing trip. This soon disappeared. About four months before admission, associated with another cold in the head, shortness of breath was again marked and there was also a sharp pain under the left breast upon deep breathing or coughing for three or four days. Following this upset her shortness of breath did not entirely disappear and similar symptoms occurred off and on until she entered the Mt. Sinai Hospital in New York under the care of Dr. Marcus A. Rothschild about the middle of January, 1926.

Dr. Rothschild has kindly sent me a summary of his findings and his opinion in regard to the patient. He finally decided upon neurocirculatory asthenia on a post-infectious basis as the best diagnosis he could make, although he was by no means satisfied with this diagnosis. An X-ray of the chest at that time showed the lungs to be clear and the left ventricle of the heart somewhat rounded but without definite enlargement of the heart. An electrocardiogram was normal except for an inversion of the T wave in the third lead.

Following her discharge from the Mt. Sinai Hospital she took a short rest and then returned to her work in New York as manager of a tea room.

About six weeks before admission to the Brigham Hospital with another "cold" the shortness of breath returned and also a pain in the left side of the chest. She gave up work and went to visit relatives but the shortness of breath became so pronounced that she was unable to walk more than a few steps without becoming markedly short of breath. Also her appetite became quite poor and she vomited several times. On three occasions a short time before admission she practically fainted on rather slight provocation. During these months she had lost from 15 to 20 pounds in weight.

Physical examination upon entrance at the Brigham Hospital was essentially negative except that upon such slight exertion as talking or moving in bed she became cyanotic and exceedingly short of breath. She was able, however, to lie flat comfortably. Her blood pressure was 92 systolic and 68 diastolic. Her hemoglobin by the Dare method was 80 per cent; red blood count 5,630,000; white blood count 14,350. The differential leukocyte count showed polymorphonuclear neutrophiles 70 per cent; lymphocytes 23 per cent; large mononuclears 6 per cent; and eosinophiles 1 per cent. In the smear the red blood cells appeared normal. Subsequent examinations showed that the leukocytosis increased slightly. The urine was essentially negative on several examinations except for a moderate number of leukocytes, the origin of which was not investigated. The stools were normal and the blood Wassermann reaction negative. A basal metabolism study on April 17 showed +5 per cent. Her vital capacity on April 20 was only 53 per cent of her theoretical normal. One physician who examined her thought that the right side of the chest moved more freely than the left and that there was slight dullness and a few crackling rales in the upper left front. The house physician made the preliminary diagnosis of neurocirculatory asthenia.

On March 31, 1926, Dr. L. B. Morrison of Boston reported upon the X-ray study of her lungs by stereoscopic plates. He found increased fibrosis at the hilum of both lungs and quite a bit of fibrosis at the cardiohepatic angle. He

also found some thickened pleura at the very peak of the left diaphragm without any free fluid. He thought that there had been some subacute inflammatory process at that point which had caused a peribronchial thickening and a thickened pleura and that it was subsiding. There was no evidence of tuberculosis.

On April 20, 1926, a seven-foot film of the heart taken at the Brigham Hospital showed a definite enlargement in the region of the left ventricle. The internal diameter of the chest was 22.5 cm.; the right border of the heart 4.4 cm. and the left border 9.6 cm. from the midsternal line. The shadow of the great vessels above the heart was 5.5 cm. wide.

During her stay in the hospital her temperature was not elevated. Her pulse rate was with few exceptions between 100 and 110 per minute and it did not become appreciably faster as death approached. Her respirations at rest ranged from 20 to 30 per minute.

An electrocardiogram showed a right ventricular preponderance with an inverted T wave in both the second and third leads. During her stay of nine days in the Brigham Hospital the shortness of breath upon exertion, no matter how slight, became more marked. She became gradually more cyanotic upon exertion and even at rest, and finally somewhat orthopnoeic. She also developed exhausting coughing spells. Her pulse became gradually weaker in volume. On the morning of her death as she was markedly cyanotic and struggling for breath one of the attending physicians remarked that had the symptoms developed suddenly it would be the typical picture of an embolus in the pulmonary artery.

Autopsy Report

A postmortem examination was made eight hours after death without opening the skull. From the heart's blood a streptococcus hemolyticus was obtained in culture but as no signs of its activity were seen in the lungs or other organs its presence was not considered of importance. Although the important findings were in the lungs and heart a few points of interest were found in other parts of the body and will be mentioned first. The organs that are not mentioned were essentially normal both in the gross and under the microscope.

Slight passive congestion was found in the liver, spleen and kidneys. In addition, in the liver there were some small areas of early focal necrosis. Also in the liver there were a few small nodules containing giant cells in some of which were small stellate bodies. Similar lesions were also present in the lungs and will be described in more detail below. In the uterine wall there were a few small leiomyomata and about the external os of the cervix there was a superficial erosion with microscopic cysts in the glands beneath. Furthermore in the left uterine vessels and their contributaries there were ante mortem clots which in some instances completely occluded the

vessels. Also in one section of the vagina for microscopic study a large adjacent vein contained a thrombus without evidence of organization or infection about it. The aorta showed only slight evidence of arteriosclerotic changes and the smaller arteries in the various organs of the body seemed in excellent condition except for those in which thrombi were present.

Heart: The heart weighed 280 gm. It seemed somewhat distended and rather large for the weight and after being severed from the great vessels collapsed, especially upon the right side. The valves and coronary arteries were not remarkable. No evidences of mural thrombi were found. The myocardium was somewhat softer in consistence than normal and a little pale but there was no gross evidence of fibrosis. The wall of the right ventricle was hypertrophied, especially toward the base where it measured 1 cm. in thickness tapering to 0.3 cm. in thickness toward the apex. In the right ventricle the trabeculae carneae were much hypertrophied. Microscopic examination of the heart was also essentially negative except for a section of the auricle which showed a thrombus which apparently was an ante mortem formation but quite recent as there was no evidence of beginning organization. This was apparently overlooked in the gross inspection of the auricles although they were carefully searched for mural thrombi.

Upon opening the pulmonary artery within the pericardial cavity only fluid blood was found in the larger branches and nothing remarkable was noted about the vessels.

Lungs: The lungs were normal in shape and pink in color except for the extreme posterior borders where they had a dark red almost purplish tinge. This darkening of the posterior margins covered a smaller area than ordinarily noted. The right lung weighed 300 gm.; the left 250 gm.

The left lung was crepitant throughout except for two small areas, one on the lower sharp border of each lobe. Here on the surface of the lung there were sharply demarcated dense white areas each triangular in shape, the height of the triangle being about 1.5 cm. and the base resting at the sharp border of the lung. This tissue was very hard in consistence and had almost a cartilaginous feel. It extended between both surfaces of the lung and the edge of the tissue occupied the sharp border. The surface here was somewhat softer than the remainder of the lung. Surrounding these triangular white areas

there was a region of crepitant dark red lung which merged insensibly into the more nearly normal pink lung about it. On section of this portion of the lung the white appearance of the surface extended completely through the central part of these areas taken to be old infarcts and about them was a border of congested firm lung tissue about 0.5 cm. in width. Sections elsewhere in the left lung showed only crepitant grayish pink tissue which however seemed to have a slight brownish tinge. It had a somewhat more rubbery feel than normal lung but was crepitant throughout. No exudate could be expressed from this lung.

In the right lung five or six infarcted areas were found in similar locations to the two in the left lung, that is along the sharp borders of all three lobes. Two of these had a similar appearance to the two in the left lung, that is the central portion was dense, almost cartilaginous. The others varied from this picture to one suggesting a very recent infarct. This latter area was raised somewhat above the surface of the surrounding lung and was dark red in color and at its border; both on the surface and on cut section it had a fairly well demarcated edge. Intermediate between these two extremes the other infarcts had a grayer center than the firm one and this central portion was softer although still quite firm. About each of them was a rim of firm congested crepitant lung tissue. Elsewhere the right lung showed a similar appearance on section to the left lung.

Dissection of the bronchi in both lungs revealed a slightly congested mucosa to which there was attached a small amount of brownish mucoid material. Dissection of the pulmonary veins revealed nothing unusual. The lesions about to be discussed were present in both lungs but much more frequent in the right. Upon opening the pulmonary arteries nothing unusual was found in the first and second order of branches peripheral to them. After the dissection of the branches of the third and fourth order was attempted practically all of them were found completely occluded by thrombi. These thrombi varied in size from small pin-head-sized crumbly grayish pink masses firmly attached to the wall to similar appearing masses 1 or 2 cm. in length and filling the whole lumen of the vessel. Propagated from these older thrombi in the direction of the root of the lung were adherent long dark red softer thrombi. In many cases the thrombi when pulled off were seen to be extending into some of the small pinhead size ultimate branches of the vessels, leading to the parenchyma

of the lung. When these comparatively fresh thrombi were torn off from the walls of the arteries they left rough surfaces which in some places were slightly ulcerated. In addition to these fresh thrombi a few very firm, very dense yellowish white thrombi were found firmly attached to the wall and almost compeltely filling the lumen. These latter thrombi could not be very readily crushed in the fingers, although most of the grayish pink ones could be so crushed. Other portions of the lining of the pulmonary arteries were somewhat roughened and had a yellowish white appearance although no thrombi could be rubbed from the surface. This was most marked in the smaller branches and in several places a small ring of this roughening and thickening was found surrounding one of these ultimate pin-point branches.

Microscopic Examination

For microscopic study sections were made from twenty-three blocks of the lung tissue. In all there was a very considerable increase of connective tissue in the alveolar walls and about blood vessels and bronchi. In some regions the alveolar walls were almost avascular. The alveoli throughout the lungs contained numerous mononuclear cells, most of which contained carbon pigment. Adjacent to blood vessels, bronchi, pleura and interlobular septa were tubercle-like lesions composed of mononuclear cells and giant cells (Figs. 1 and 2). Most of these lesions contained numerous lymphoid and plasma cells and some showed beginning fibrosis. In the giant cells were found the stellate inclusions described by Wolbach [15] in 1911, and the histology and distribution of the lesions in the lung were in general identical with those described by him. The lesions in the liver with giant cells mentioned above probably represent the same process.

The infarctions noted in the gross showed varying degrees of repair. Some of them consisted essentially of rather dense cicatricial tissue but the majority showed necrotic centers, preserving roughly the architecture of the lung parenchyma and surrounded by dense fibrous tissue. There were a few recent infarctions with hemorrhagic peripheral zones undergoing organization. None of the infarctions showed evidence of suppuration.

The important pathology of the lung concerns the blood vessels. Many of the smallest recognizable arteries showed an extraordinary

thickening of their walls and were composed of a dense connective tissue with a very narrow lumen lined with swollen endothelial cells. Here and there these vessels showed acute change, on the whole reminiscent of the lesions found in the glomerular arterioles in chronic nephritis accompanied by hypertension (Figs. 3 and 4). The smooth muscle layer was intact but the media greatly thickened and sparsely infiltrated with mononuclear cells (macrophages). The lumen in most instances was almost completely obliterated by compression and lined with swollen endothelial cells. The thickened intima was characterized by a lightly staining collagen and by the presence of fairly well preserved red blood corpuscles, as if both the endothelium and connective tissue had become permeable to particulate matter.

These acute lesions were comparatively few in number and concerned vessels of the diameter of 0.03 mm. to 0.05 mm. Subsequent sections in the vascular lesions were difficult to follow. It was quite evident that complete occlusion of some small arteries had resulted. In the majority of instances the repair had been accomplished by a new deposit of collagen producing concentric or eccentric thickening of the blood vessel wall and throughout the sections studied there were found small arteries showing early and late cicatricial thickening of the intima (Fig. 5). In larger arteries there had evidently been organization of thrombi inasmuch as many were found containing several endothelial-lined channels. In arteries regarded as showing the oldest lesions thickened walls were frequently found to contain cells resembling in all respects smooth muscle cells such as are found frequently in old canalized thrombi (Fig. 6). In arteries of larger caliber, of dimensions in the neighborhood of 1 mm. to 1.3 mm. there were found completely organized and canalized thrombi. The channels were lined with endothelium and backed up by smooth muscle cells; these represented the late stage of repair (Fig. 7). Such canalized arteries on the whole show a practically normal media and a normal distribution of elastic tissue. Fresh thrombi and thrombi with early organization were found only in arteries of considerable size, those with a diameter of 1.5 mm. and over (Fig. 8).

In these larger arteries the histology gave no clue to the causation of the thrombi. On the other hand, the microscopic findings supported the conclusions made at the time of the postmortem that these fresh thrombi were propagated from the more distal organized

and canalized thrombi. In none of the sections were arteries found with unusual degrees of the arteriosclerotic or atheromatous processes. Only in arteries in close proximity to infarctions were acute changes simulating the early stages of atheromatosis found.

Discussion

While in the study of these lesions many details were noted in the histology of the blood vessel lesions, no completely satisfactory selection of lesions could be made in the attempt to work out the sequences which gave rise to the predominating pathological condition, *viz.*, a propagating thrombosis with organization and canalization.

It seems warrantable to conclude that the acute lesion, consisting in swelling and cellular infiltration of the vessel wall represents an early if not the initial damage. The infiltrating cells for the greater part were mononuclear cells (macrophages). Polymorphonuclear leukocytes were practically absent. Eosinophiles and mast cells were occasionally present. The presence of mononuclear cells together with the swollen endothelium permits the consideration of the possibility of an infectious agent as causative factor.

A wholly warrantable conclusion is that the oldest lesions as well as the most recent are to be found in the minute arteries and that the thrombosis traveled by centripetal progagation. The explanation of the continued propagation of thrombi over the long period indicated by the clinical history and by the histological findings presents a problem not solvable by the histological findings, because the type of lesion seen in the smallest arteries is not present in the larger arteries with recent thrombi, nor is there anything in the appearance of the organized and canalized thrombi to account for the continued propagation.

An hypothesis which may be entertained is that the lungs in this case had been the seat of an extensive interstitial pneumonitis. This hypothesis is suggested by the generalized fibrosis and by the tubercle-like lesions adjacent to bronchi, blood vessels and connective tissue structures of the lung, all regions of lymphatic channels. In fact it is quite evident that many of the smaller tubercle-like lesions have arisen in lymphatics. Whether or not the initial vascular damage was done during the period of interstitial pneumonitis cannot be solved, but it would seem reasonable to accept this explanation

rather than to assume the presence of an unknown infectious agent of unique behavior.

On the whole, the closest analagous vascular pathology is to be be found in the disease known as thrombo-angiitis obliterans. Aside from the distribution of the lesions, the similarity of the process to the present case seems evident. At least there is in both conditions centripetally propagated thrombosis of arteries accompanied by organization and canalization. The pathology of the arteries in this case is more nearly expressed by the term endarteritis or arteritis than by arteriosclerosis or atheromatosis.

It is not evident whether the thrombosis in the uterine vessels and the microscopic thrombus found in the auricle were due to the same process as the lesions in the pulmonary arteries. The lesions in the pulmonary arteries were evidently much older.

In this case it is interesting to note that with these multiple small infarctions of all ages in the lungs very little evidence of them was found upon X-ray examination. It is also interesting to speculate whether the so-called fresh "colds" and periods of shortness of breath represented periodic advancement of the thrombus formation, or recurrence of the acute arterial lesions. In the cases cited from the literature, even those by Eppinger and Wagner which strongly suggested the lesions found in this case, the outstanding symptoms usually were cardiac failure. In this case the heart muscle had not given way to the extent of the production of edema and pronounced passive congestion, but already evidence of hypertrophy of the right ventricle had appeared and it is possible that the lack of symptoms of cardiac failure could be explained by the fact that the patient died early in the course of the disease.

The lesions in this case are certainly different from ordinary arteriosclerosis, also from lesions due to syphilis and also from some of the cases of thrombosis of the pulmonary arteries that have been reported. The lesions may be similar to those in a few of the cases with thrombosis of the pulmonary arteries and cardiac failure that have been reported. Possibly the case may represent a unique lesion of the pulmonary arteries.

Summary

This case clinically during life presented symptoms of unexplained shortness of breath and cyanosis upon exertion gradually increasing

over a period of months. The cause for it became apparent at autopsy as due to thrombosis of the smaller branches of the pulmonary arteries with resulting infarctions and injury to lung tissue.

This thrombosis began in the smallest branches of the pulmonary arteries and propagated centripetally toward the larger branches.

The cause for the beginning of the thrombosis or its tendency to propagate was not apparent.

In the walls of some of the smallest branches of the pulmonary arteries were slight acute lesions for which the cause was not apparent.

The relation of the tubercle-like lesions in the lung to the vascular lesions is unsettled.

Note: For the photomicrographs and many helpful suggestions I am indebted to Professor S. Burt Wolbach.

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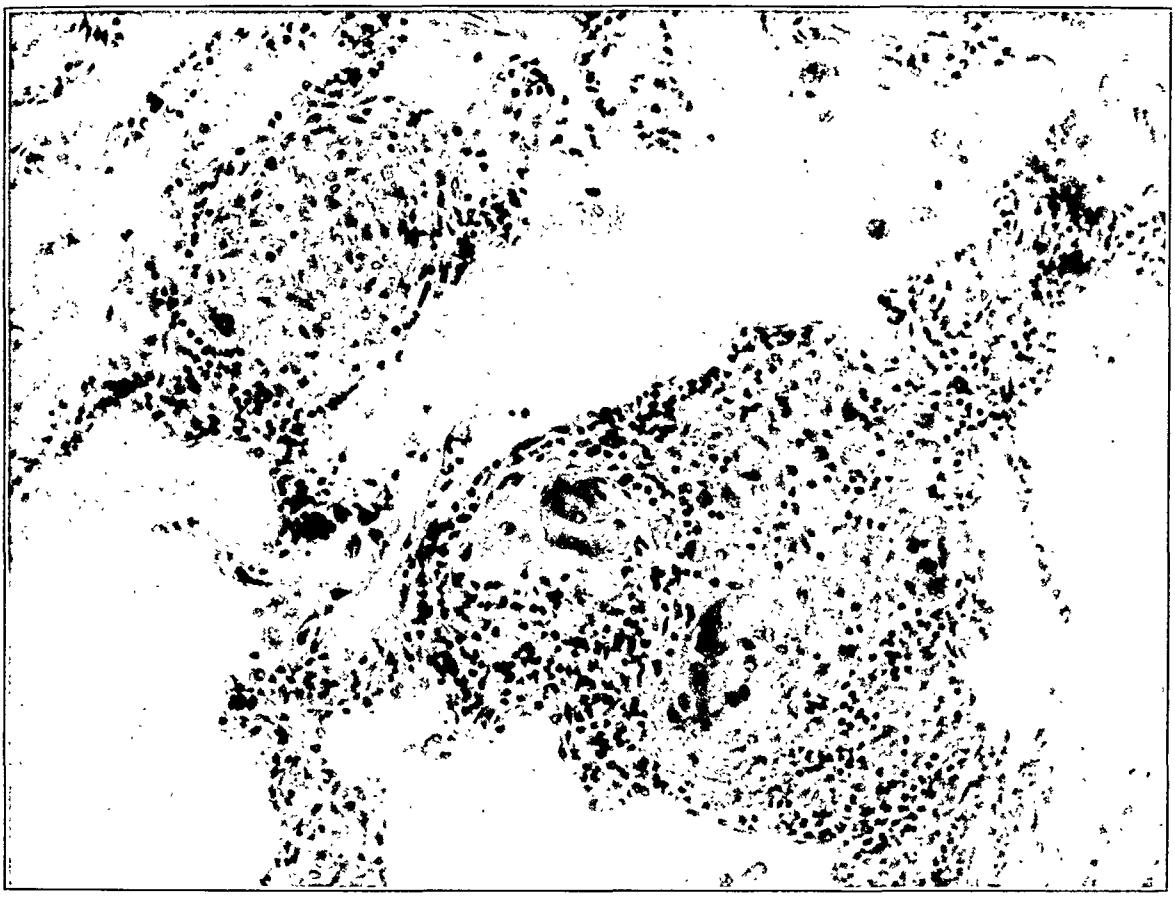
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DESCRIPTION OF PLATES

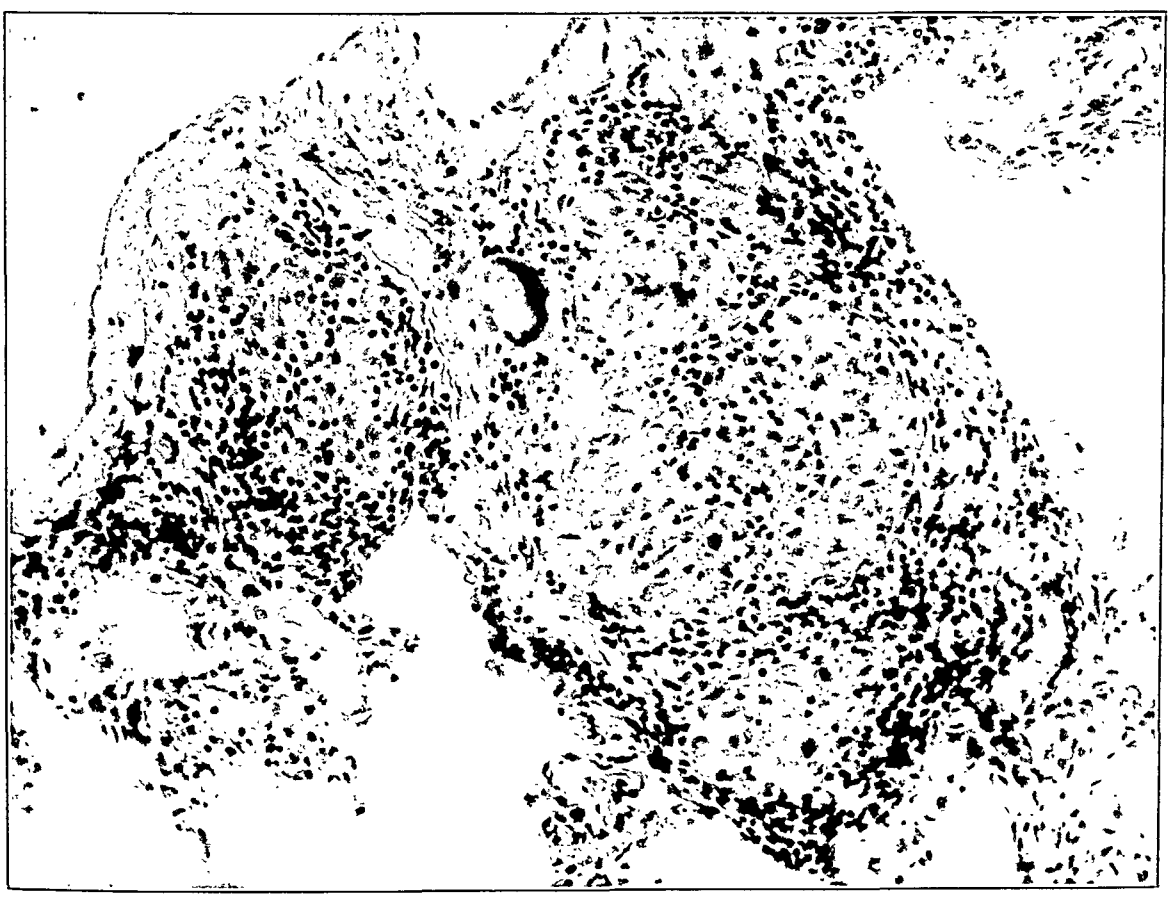
PLATE 5

FIG. 1. Tubercle-like lesion in the lung showing giant cells. × 210.

FIG. 2. Tubercle-like lesion in the lung showing giant cells. × 210.



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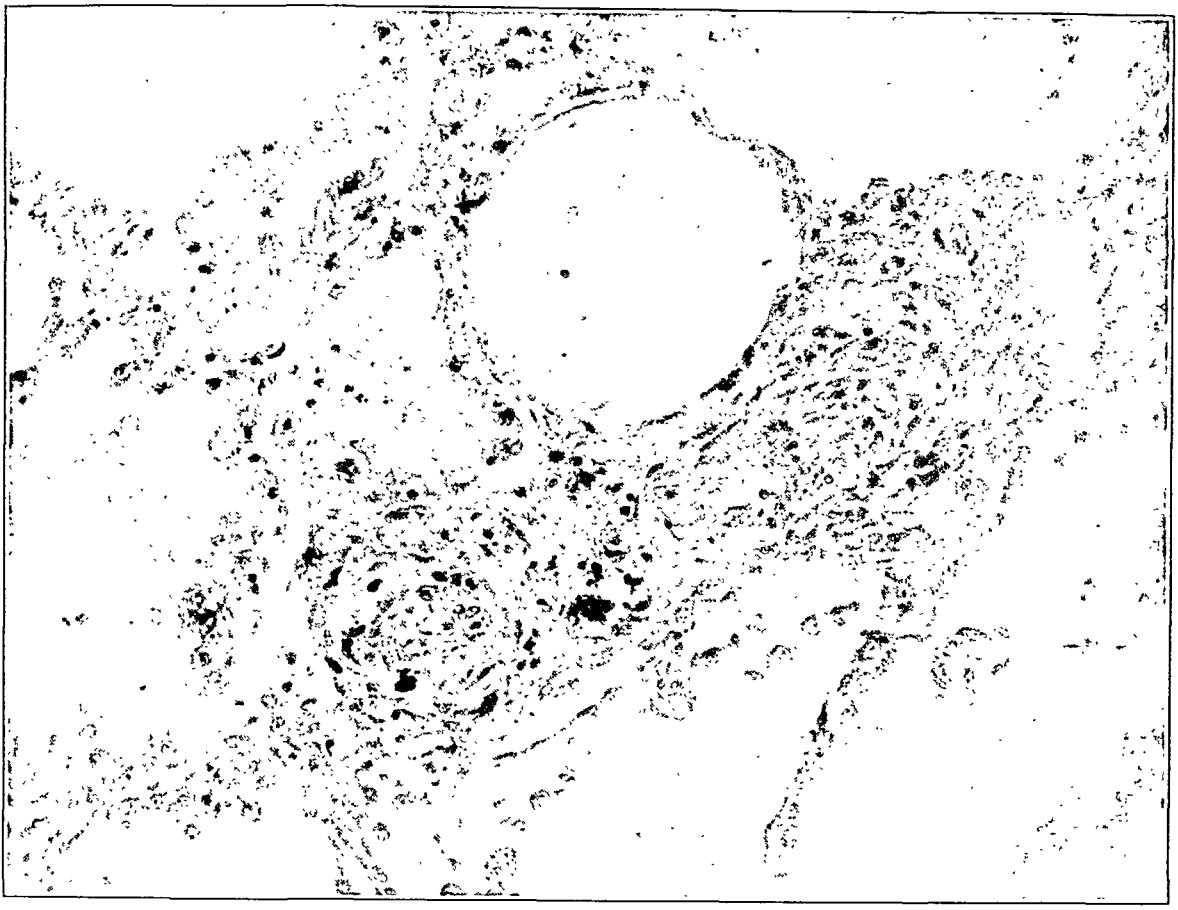
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Frothingham Thrombosis of Branches of Pulmonary Arteries

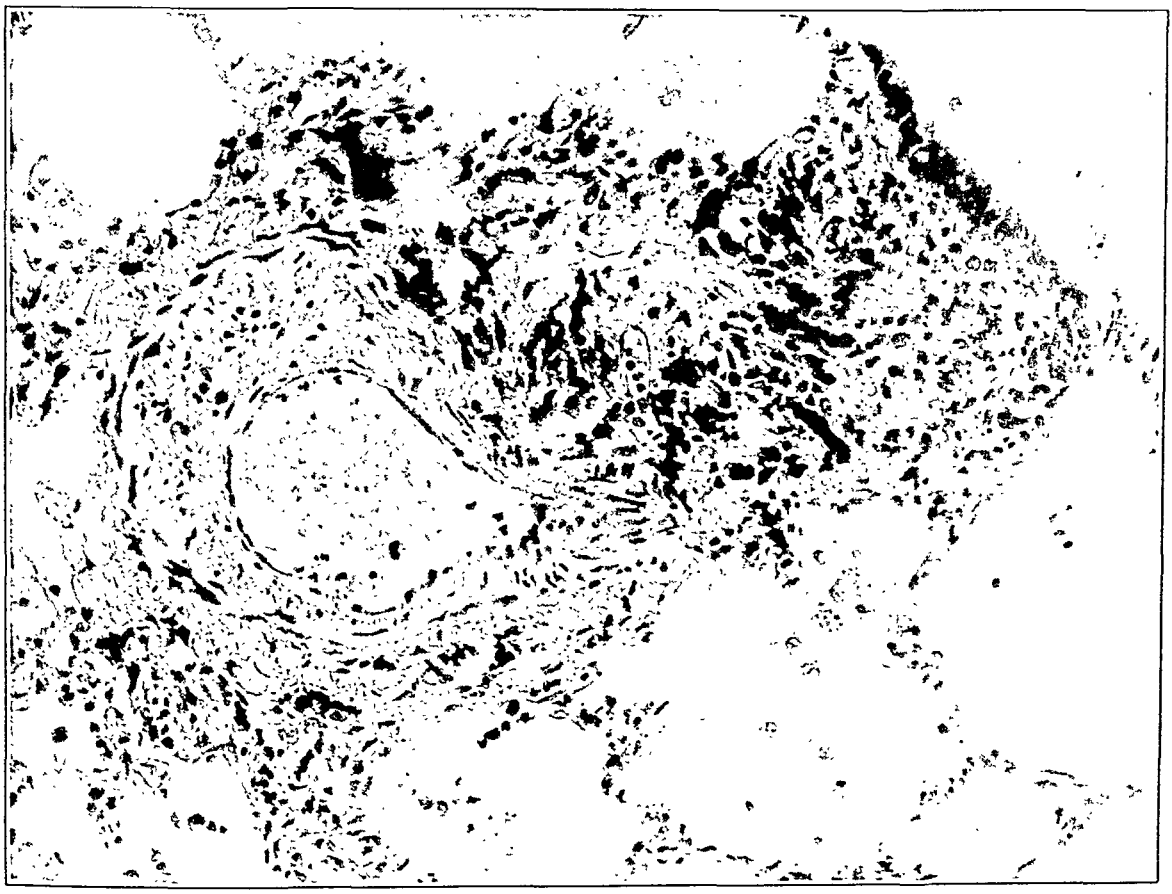
PLATE 6

FIG. 3. Acute vascular lesion showing thickening of the vessel wall with swelling of the endothelium and invasion with mononuclear phagocytes. × 210.

FIG. 4. Acute vascular lesion showing thickening of the vessel wall with swelling of the endothelium and invasion with mononuclear phagocytes. × 210.



3



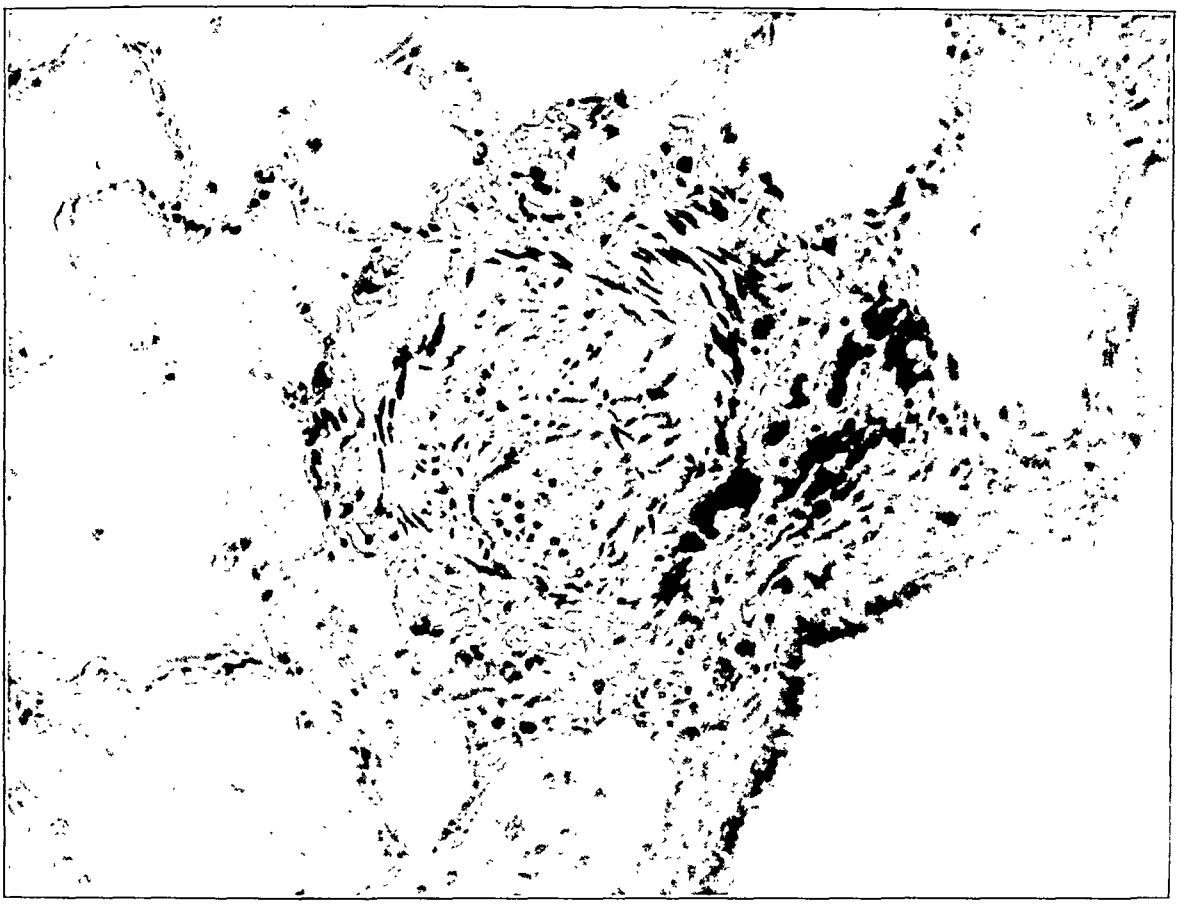
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Frothingham Thrombosis of Branches of Pulmonary Arteries

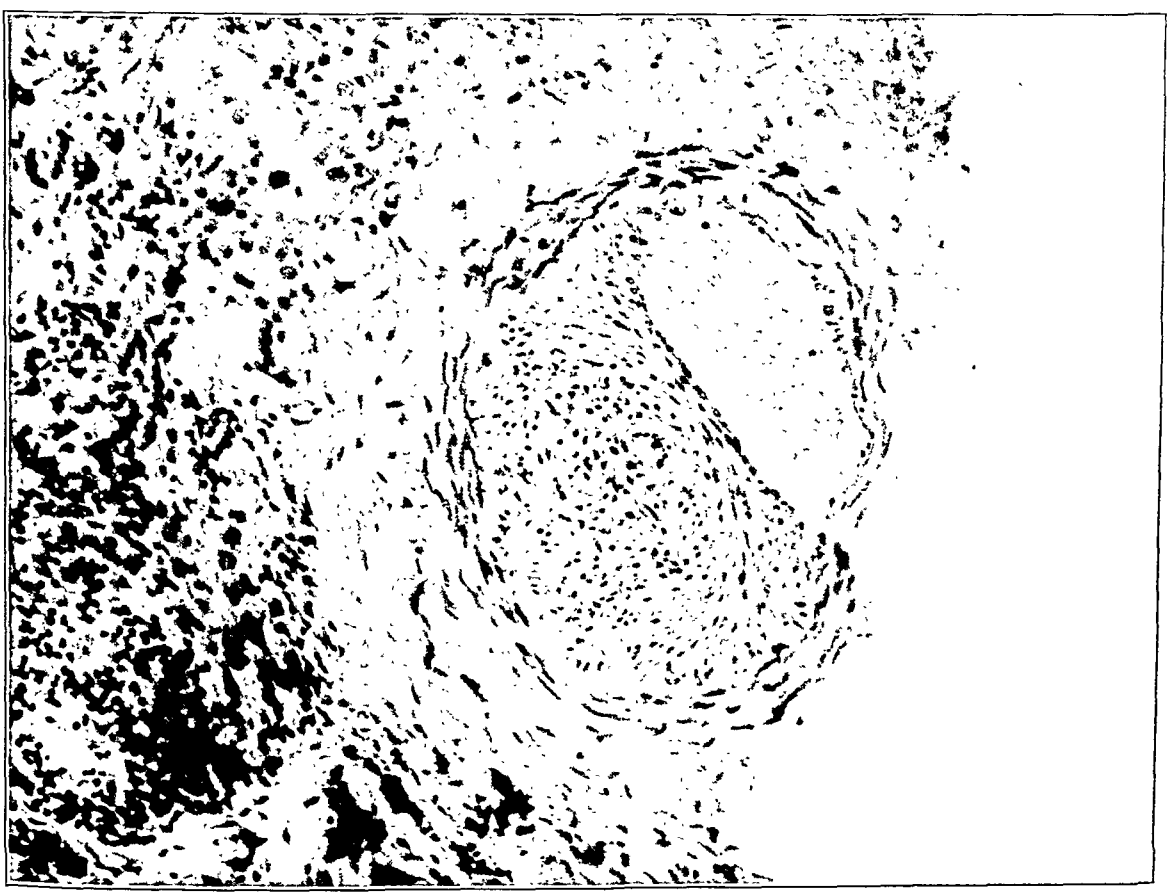
Plate 7

Fig. 5. Thickened blood vessel wall with connective tissue, either endarteritis or an organized thrombus. × 210.

Fig. 6. Connective tissue narrowing the lumen of an artery with cells suggesting smooth muscle cells, presumably an old organized mural thrombus. × 210.



5



6

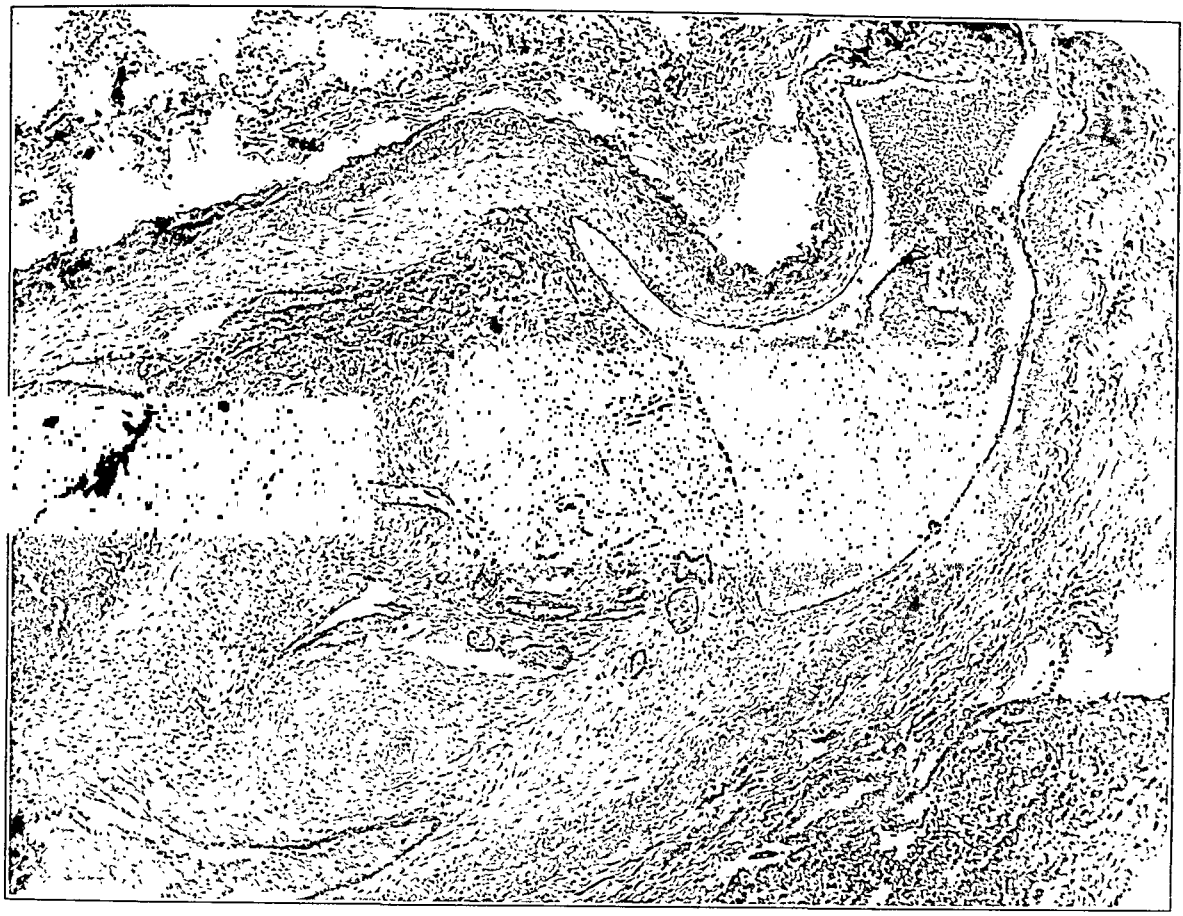
Frothingham

Thrombosis of Branches of Pulmonary Arteries

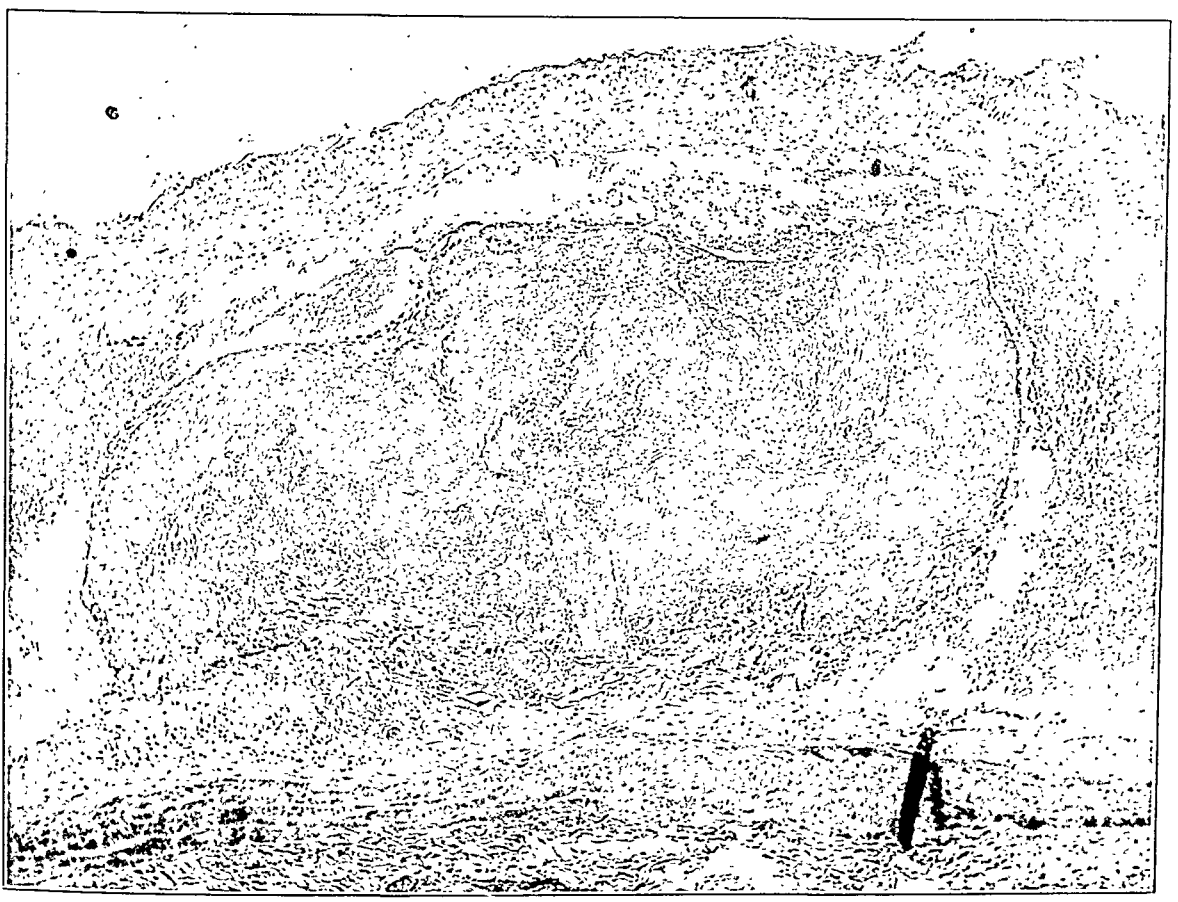
PLATE 8

FIG. 7. Thrombus in an artery with canalization in the thrombus. × 62.

FIG. 8. Fresh thrombus with early organization in an artery. Arterial wall in good condition. × 62.



7



8

Frothingham Thrombosis of Branches of Pulmonary Arteries

A CASE OF MAMMARY GLAND TISSUE IN THE AXILLA*

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In the monograph upon "The Breast: Its Anomalies, Its Diseases and Their Treatment" by Deaver and McFarland,[1] some ninety-five publications recording more than a hundred cases of axillary mammary tissue are reviewed, and arranged as follows:

I. Cases in which lumps of painful character appear in the axilla during pregnancy or at the beginning of lactation, either where small painless lumps have existed since puberty, or where the patient was unconscious of the existence of any abnormality.

II. Cases in which "axillary lumps," similar to those mentioned, occur under like conditions, but discharge a milky secretion through openings of varying size and number.

III. Cases in which the "axillary lumps" are surmounted by a more or less distinct areola with one or more rudimentary nipples.

IV. Cases in which there is a distinct mammary gland in the axilla, upon which there is a distinct areola and a fairly well formed nipple.

The following case seems to belong in Group I of this tabulation, that in which the greatest number of incidences occur.

REPORT OF CASE

The patient is a young married woman, 23 years of age, who two years ago gave birth to her first child. At the usual time after delivery the breasts swelled and a lump occurred in the right axilla that quickly grew to the size of a hen's egg.

A binder was applied to the breasts, which diminished in size, and at the same time the lump in the axilla grew smaller although it never went away.

Not long ago the patient spoke of having a "lump under her arm," and her brother-in-law, who is a surgeon, became interested and suggested an examination at which another similar but smaller "lump" was found in the other axilla. The surgeon now became apprehensive, fearing that they were enlarged lymph nodes, and possibly indicative of beginning disease of the lymphatic system.

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The lump in the right axilla caused a visible swelling when the arm was extended, and when carefully palpated seemed to be about 4 by 2 cm. in size and resolved itself into three rounded lobules or portions, one free and two connected like a dumb-bell. All were insensitive and movable, except for intimate connection with the skin. The patient consenting, the larger "lump" was removed under local anesthetic and no enlarged lymph nodes were found. Incision of the excised tissue resulted in the escape of whitish fluid that looked like milk.

Microscopic examination revealed a histological structure corresponding to that of the mammary gland.

This led to further interrogation of the patient, from whom it was learned that the lumps in the axillae enlarged and became a little tender at each monthly period but that they never "leaked" or discharged any milky fluid either at the time of the pregnancy enlargement that led to their first observation, or at any time since.

Resemblance between the large sudoriparous glands of the axilla and mammary tissue have sometimes caused the former to be mistaken for the latter. The coiled tubules of the eccrine glands do resemble the smaller lobules, and the broad lumenated apocrine glands suggest the "residual secretory acini" of the mamma..

Inasmuch as both the sudoriparous and lactiferous glands are so closely related in structure that the latter is looked upon as an exaggerated and specialized homologue of the former, there is every reason that the histological resemblances should be close.

Each sudoriparous gland seems to consist of a single tubule (it is said that in some of the larger glands the tubule may give off a branch), while a mammary gland is composed of a highly complex system of branching tubules. In each there is a secretory and a conducting portion whose structure varies with its function. The secretory portion of each tubule of each gland has the same theoretical fundamental structure, and consists of a membrana propria surmounted by two layers of cells, the outer and less conspicuous sometimes known as the "basket cells" being regarded as ectodermal involuntary muscle cells, the inner much larger and cuboidal, known as the "chief" or secretory cells. The prominence of the latter as compared with the former, sometimes gives the false impression of tubules lined by a single layer of cells. Again, and especially in the mammary glands, the contraction of the muscle cells seems to cause

crowding of the secretory cells, to disguise their relation to the basement membrane and to confuse the cells of the two layers so as to give the impression of several layers of cells with no limiting membrane. As the ever increasing quantity of secretion requires larger and larger tubules to accommodate it, the tubules of the lactiferous glands unite with others, and still others, as they approach the nipple at which their common outlets are situated, eventuating in large ducts lined with columnar epithelium, and terminating in ampullae.

The tubules of the sudoriparous glands receiving no accessions, remain approximately of uniform diameter except that during active secretion they dilate widely. But as the secreting part of the tubule ends and the conducting part is reached, instead of expanding as is the case with the lactiferous glands, it diminishes to about one-half its former diameter, loses its muscle cells, and develops a double or treble cell layer with a homogeneous zone or cuticle about the lumen.

The larger apocrine glands are less compact, the lumens are wider, the epithelium more flattened, and the cytoplasm of the cells distinctly eosinophilic.

But apart from these simple histological considerations, there is a remarkable difference in behavior that is based upon the respective functions of the different glands.

The sudoriparous glands although not constantly active, must ever be ready to assume their important functions, and, therefore, have a structure that may be looked upon as permanent and invariable, while the mammary glands are only occasionally called upon to exercise their function, for which there must be elaborate preparation. Their structure, therefore, varies from time to time to an extraordinary degree, accordingly as they are resting, or in process of evolution or involution.

The outset of estruation in animals, and menstruation in humans, is marked by phenomena that vary in extent in different animals, and in degree in different individuals. It is now pretty well agreed that these are the result of the exciting effects of a hormone originating in the ovary and probably derived from the liquor folliculi or secreted by the corpora lutea.[2]

There is at present no suspicion that this hormone in any manner affects the histology of the sudoriparous glands, but its effect upon the lactiferous glands is striking, for their previously quiescent

tubules begin to extend and branch until distinct lobules of mammary tissue are formed.

As it is at estruation that the sexes usually unite and, therefore, at that time that conception takes place, it might not be wide of the mark to conclude that this growth of mammary tissue is in anticipation of expected gravidity, and to gain time for the voluminous increase of tissue that must nourish the resulting offspring. If no conception follows, the termination of estruation is followed by atrophy of the useless glandular tissue, and the return of the gland to its "resting stage."

The application of this knowledge to what takes place in the axillae of those rare cases in which women have lactiferous gland tissue in that situation, is sufficient to explain the clinical phenomena. During virginity they may never suspect such an anomaly, but when pregnancy occurs, the gradual growth of all lactiferous tissue may result in the appearance of a "lump" wherever such tissue is situated, and when lactation sets in, any such "lump" may undergo a sudden and painful increase because of the secretion and retention of the milk. If the milk escapes or can be expressed the pain is relieved; if that cannot be done further painful enlargement may occur and become so severe as to necessitate operation. In ordinary cases the pain soon subsides and the lump gradually diminishes, sometimes disappearing altogether. But in many cases the patient can find it by palpation, and finds it sometimes sensitive at the menstrual periods, because of the slight hypertrophy of the lactiferous tissues at that time.

In the present case knowledge of the histological cyclical variations in lactiferous tissue may be of assistance in distinguishing between the sudoriparous and lactiferous tissues where they come into close juxtaposition, as is so often the case in the axilla. The biopsy, at which the tissue obtained for microscopic examination was secured from the patient, was performed on the first day of a menstrual period and only one hour before the flow began. At that period each unit of the lactiferous tissue should be surrounded by a loose zone of periductal tissue, and should be giving off those auxillary ductules or acini that form the periodically appearing lobules.

As a matter of fact, precisely that condition obtains. There is proliferation of the glandular tissue, and some secretion fills the ducts of the mammary tissue.

It seems, therefore, that the clinical history of the case, the surgical discovery of a collection of milk at the time of the operative removal of the tissue, and the histological findings all point clearly to this case as one of mammary tissue in the axilla.

REFERENCES

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2. Rosenberg, A. *Frankfurt. Ztschr. f. Path.*, 1922, xxvii, 466.

DESCRIPTION OF PLATE

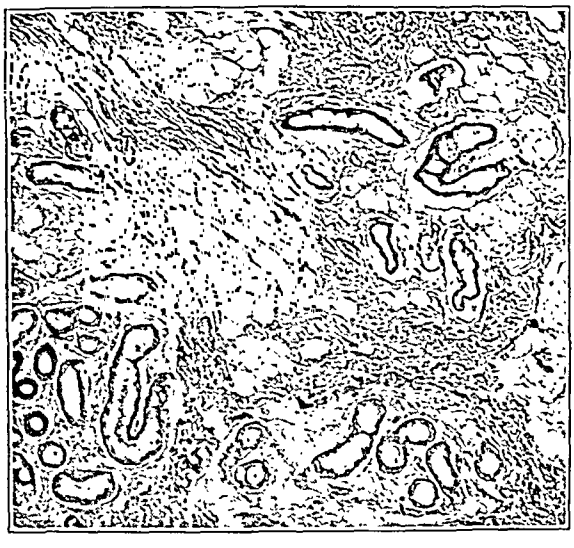
PLATE 9

FIG. 1. An eccrine gland below on the left, apocrine glands elsewhere. This is from the superficial part of the removed tissue.

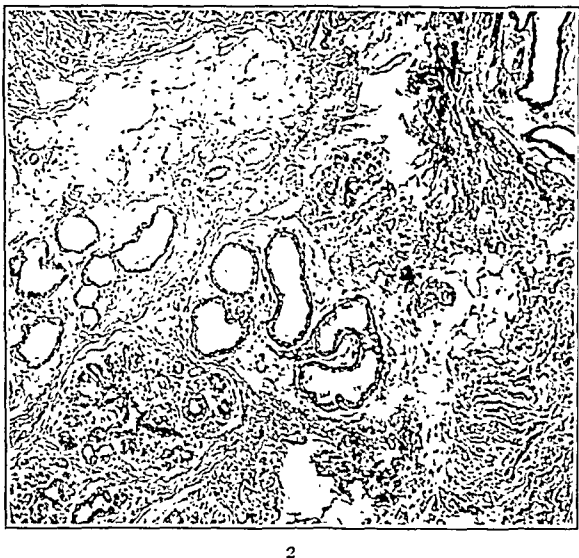
FIG. 2. Below and to the left three mammary lobules, two partly blended, the third separate. Elsewhere apocrine tubes of sudoriparous glands.

FIG. 3. More deeply in the removed tissue one finds mammary tissue only.

FIG. 4. Various lobules of mammary tissue in the deeper part of the removed tissue.



1



2



3



4

MALIGNANT THYMOMA WITH METASTASES *

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The case of malignant thymoma reported here presents clinical and pathological features of unusual interest and our observations are recorded with the hope of contributing information that may aid in arriving at a correct diagnosis in an early stage of a condition heretofore recognized only in the advanced form and then only after biopsy and in the majority of cases only after autopsy.

Throughout the early months of our case, we met many diagnostic difficulties which were augmented rather than lessened by recourse to laboratory assistance and it is believed that an accurate presentation of our observations will tend to a better understanding of the problems met in thymic neoplasms of a malignant nature.

The term thymoma is misleading because it simply implies a tumor, benign or malignant originating in the thymus gland; however, since that appellation was first proposed by Ambrosini [1] in 1894 it has been generally adopted to describe an atypical malignant neoplasm of the thymus gland.

The pathology of a thymoma is still a matter of controversy and will remain so until histologists agree upon the normal constituents of the thymic parenchyma. A satisfactory discussion of this subject entails a review of the embryology, histogenesis and pathology of the thymus gland and as that has been presented in recent writings by Foot,[2] Jacobson,[3] and Symmers and Vance,[4] it seems superfluous to enter such a discussion here. We will, however, refer to these articles where points relevant to our case are encountered.

In describing the disease entity called thymoma we quote Ewing's [5] description, not only because he is a recognized authority on the subject but also because he was a consultant in our case. According to him a thymoma is a lymphosarcoma of the thymus gland, composed of thymic or reticular cells, occupying the anterior mediastinum extending from the sternal notch or as high as the thyroid gland down to the diaphragm. A thymoma usually surrounds

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and compresses the trachea, bronchi, pericardium and great vessels. By compression and occasionally by invasion of vessels and air passages death is caused by asphyxia and venous obstruction which may be gradual or sudden. The tumor mass is encapsulated within the mediastinum in some cases but in others it becomes adherent to surrounding organs and invades the pleura, lung, pericardium, walls or lumina of vessels, trachea, bronchial, cervical and axillary lymph nodes. Metastases to distant organs are occasionally noted involving spleen, liver, adrenals, pancreas, kidney and bone marrow of the humerus (Zniniewicz).[6] In our case there were also metastases to the stomach and to one ovary. Strauss[7] reports a case in which the tumor invaded the musculature of the auricles and completely obliterated the lumen of the vena cava by pressure. The pericardium was involved in our case but the myocardium escaped invasion.

Thymoma is an uncommon neoplasm and only about ninety cases have been reported, which include those in which neither biopsy nor autopsy were done and hence the accuracy of the diagnosis may be questioned. The sixty-nine cases of tumor of thymic origin which Rubaschow[8] collected from the literature include both sarcomas and carcinomas and it is doubtful that all these cases would be classed as thymomas in the light of more modern research. Numerous cases have been reported with distant metastases yet only three (Ambrosini,[1] Symmers and Vance,[4] Friedlander and Foot[9]) are on record in which the process invaded organs below the diaphragm and ours makes the fourth.

Although the literature on thymoma is quite extensive but little reference is made to its clinical phases, most of the discussions being confined to the pathological findings. In searching the literature it was hoped we would receive some enlightenment from the clinical experiences of others that might aid in making an early diagnosis but in the majority of cases heretofore reported no statement is made whether the correct diagnosis was arrived at before resorting to biopsy. We note, however, that some observers met with diagnostic experiences similar to ours and it is of interest to compare them. Foot and Harrington[10] reported a case of thymoma in which the original diagnosis was unresolved pneumonia. Miller[11] records a patient whose diagnosis lay between a laryngeal diphtheria, heart disease, a foreign body in the air passages, asthma and some vague

intrathoracic growth. Jacobson [3] in reporting a case of primary neoplasm of the thymus emphasizes the difficulty of an ante mortem diagnosis. His case was originally diagnosed muscular rheumatism by a local physician and later a surgeon considered the condition one of metastatic cancer of the spine. Apparently the early manifestations directed attention to the metastatic condition rather than the primary focus. Even the roentgen-ray examinations in this case were misleading for they pictured a process of the spine simulating Pott's disease and metastatic infiltration of the lungs. To cite another example of the difficulty of an early diagnosis we mention the experience of Helvestine [12] who reports a case in which the first diagnosis was intrathoracic tumor with tumor of the neck, possibly a combination of Hodgkin's disease with exophthalmic goiter. Our case presented similar diagnostic difficulties and only after repeated biopsies and pathological studies was a correct diagnosis finally made. In the case we report below, the diagnoses varied with the progress of the disease and were more or less influenced by certain laboratory studies and roentgenograms. At various stages the clinical signs were those of non-toxic substernal thyroid, status thymicolymphaticus, lymphosarcoma, and Hodgkin's disease. The two former diagnoses were at first confirmed by roentgen rays, but serological and histological examinations misled us in suspecting syphilis and later tuberculosis. Subsequent X-rays however disclosed a distinct mediastinal newgrowth and the final biopsy presented the histological picture of thymoma.

Report of Case

Clinical Report: A. H., a white female of the phthisical status, aged 25 years. First seen July 27, 1927.

Chief Complaint: Swelling of neck of three weeks duration interfering slightly with breathing; some pain at base of neck.

Family History: Mother died of cancer of the stomach.

Past History: Tonsillectomy in childhood.

Present History: Noticed swelling in thyroid region of neck about three weeks ago accompanied by no other symptoms at that time, except an "embarrassing" pinkish discoloration of eyelids and brows. More recently she complained of becoming easily fatigued on exertion and feels somewhat nervous. The thyroid swelling is slowly but steadily increasing. There is a tendency to shortness of breath.

Physical Examination: Well developed, fairly well nourished female. Pale complexion with an unusual sharply circumscribed pinkish discoloration involving eyelids and brows, giving the impression of a theatrical "make up."

Eyes: Reacted normally to light and accommodation and were equal on both sides.

Nose and Teeth: Negative.

Throat: Ulcer in vault of pharynx. Tonsils, removed.

Thyroid: Uniformly enlarged but of a greater density on palpation than is usual in simple goiter or in Graves' disease. There were no nodular masses as in adenoma of the thyroid or in tuberculous glands or Hodgkin's disease.

Chest: No deformities; no pulsation; no bulging; no engorgement of veins of chest or arms. Substernal dullness 9 cm. in diameter extending from the thyroidal enlargement down to the base of the heart.

Heart: Showed no abnormality excepting that the apex measured only 7 cm. from the midsternal line. No arrhythmia, no murmurs, no thrills; a normal rate of 72 to the minute.

Lungs: Negative except for the above-mentioned dullness.

Abdomen: Liver and spleen not palpable and no other signs of abnormality.

Extremities: Negative excepting for eczema of elbows. No clubbing of fingers.

Blood Pressure: Systolic 120; Diastolic 80. No difference between right and left arm.

Superficial Reflexes: Normal.

Superficial Lymph Nodes: Not palpable. *Peripheral vessels* not thickened.

Tentative Diagnosis: Substernal, non-toxic goiter.

Laboratory Findings: To this date were essentially negative.

Progress Notes

August 11, 1927: Slight swelling of cervical glands was noted. Wassermann taken and reported negative.

August 18: Admitted to hospital for further study. Physical examination confirmed above findings except for an increased heart rate of 96 per minute with frequent premature contractions. The sounds were of good quality without murmurs. *Lungs* and *abdomen* presented no changes since previous examination.

August 20: A roentgenogram at this time showed a thorax long in its vertical diameter with the heart small and inclined to the rotate type. An increase in density in the superior mediastinal region is noted especially on the right side together with a clouding above this suggesting probable substernal thyroid.

August 24: Basal metabolism reported normal. Further study of the roentgenogram led to the belief that the substernal shadow was attributable to a persistent thymus rather than to a substernal thyroid, which fact, together with a small heart and the general lymphatic type of individual, justified the revised diagnosis of "status thymicolymphaticus."

A *blood count* done at this time gave the following figures: Reds, 5,090,000; whites 7,900; hemoglobin 87 per cent.

Differential Count: Polymorphonuclears 69 per cent; transitionals, 1 per cent; small lymphocytes 20 per cent; large lymphocytes 5 per cent.

Temperature readings varied from 99° F to 100° F.

Weight: Remained about stationary at 107 lbs. The patient was treated symptomatically and kept under observation. The shortness of breath, insomnia and general malaise continued to increase and the cervical glands gradually grew larger.

September 9: Biopsy was done on gland removed from the cervical region and the patient discharged from the hospital. Following are the results of this biopsy.

MICROSCOPIC EXAMINATION

Sections show several small lymph glands embedded in dense fibrous tissue. The lymph glands are circumscribed in part and in part the outlines are diffuse. Within the lymphoid substance there are no well demarkated follicles, but rather a picture of diffuse lymphocytosis. Scattered among the lymphocytes are numerous plasma cells and occasional eosinophilic polymorphonuclear leucocytes. The poorly demarcated lymph glands show numerous lymphocytes, plasma cells and eosinophiles infiltrating in all directions throughout the surrounding fibrous tissue. The fibrous tissue extends into the attached fatty tissue with infiltration by lymphocytes, plasma cells and eosinophilic leucocytes. There is a large necrotic area, chiefly in the fibrous tissue. In this necrotic area remnants of cell nuclei can be seen and occasional blood vessels and some necrotic portions of lymphoid tissue.

The striking thing in the fibrous tissue is the thickening of the walls of many blood vessels with perivascular infiltration by lymphocytes and plasma cells. The presence of numerous plasma cells and eosinophilic leucocytes together with the fibrous tissue proliferation and especially the type of necrosis, suggest a syphilitic lesion.

Diagnosis: Chronic lymphadenitis and perilymphadenitis with necrosis suggesting syphilis. In the absence of definite evidence of a newgrowth, it seems more than likely that syphilis is the proper diagnosis. We would suggest a further Wassermann test be made, preferably on the spinal fluid.

Pursuant to the suggestion of the pathologist another blood Wassermann test was made which was again negative. A similar test on the spinal fluid was not obtained. In view of the conflicting information thus obtained it was deemed advisable to send a biopsy specimen to another pathologist and Dr. Ewing was consulted. His expressed opinion follows: "The picture *resembles* tuberculosis but must be *interpreted* as a distant effect produced by radiation employed in the thymic region." For example, it is common, according to Dr. Ewing, for axillary lymph nodes to show a picture simulating tuberculosis when radiation has been applied to cancer of the breast. Accordingly a revised pathological diagnosis was rendered of lymphadeniasis with necrosis, produced by the distant effect of radiation.

Previous to receiving this report we had clinically excluded tuberculosis which conclusion had been subsequently confirmed by the X-ray findings.

September 21, 1927: A provocative salvarsan was followed by a negative Wassermann.

September 25: The substernal mass subjected to one X-ray treatment.

September 26: Vernes' flocculation test for syphilis was negative.

September 28: Patient was again admitted to the hospital for further observation and for X-ray therapy. The only complaint now being stiffness and a sense of soreness in the neck upon arising in the mornings. There had occurred a loss of 3 lbs. in weight during the last six weeks. The temperature continued to hover around 99° F and 100° F and it was deemed advisable to postpone further X-ray therapeutics until the fever subsided.

October 5: A Vernes' flocculation test for tuberculosis gave a report of 80 and 83 milligrams of flocculation per 1 cc. of fluid with a note from the technician stating that such high results denoted active tuberculosis. This observation in conjunction with the pathologist's previous diagnosis of tuberculosis after studying the first biopsy specimen together with the developing lung involvement led us to suspect an acid-fast infection. Repeated sputum examinations however failed to discover the presence of tubercle bacilli.

October 8: An annoying cough with some pain in right chest developed. Examination of the chest revealed dullness at the base of the right lung posteriorly with feeble yet audible breath sounds and a diagnosis of pleurisy was made. A roentgenogram reported a marked reduction in the size of the tumefaction in the retrosternal space though tumefaction extending downwards from the cervical region is still noted. The picture however has been altered by the addition of a low-grade bronchopneumonic or bronchiectatic infiltration involving the right lower lobe. There is also pleuritic involvement with slight exudate, probably plastic in type obscuring the right costophrenic sinus. The heart is of the mitral stenotic type, relatively small in its total area with a marked straightening of the left heart border. The roentgenological diagnosis being, "probable thyroidal enlargement; reduction in size of substernal mass; low-grade bronchopneumonic process lower right lung with pleural involvement at right base."

October 14: The dyspnea now caused decided distress and the clinical signs of pleural effusion were unmistakable.

Roentgenological examination was reported as follows: Examination of the thorax shows marked mediastinal infiltration with a low-grade bronchopneumonic process pushing into the left central lung field as well as toward the right base. On the right side the lung condition is partly obscured by concomitant pleural thickening, both interlobar and basal, along with a considerable amount of exudate obscuring the lower half of the right pulmonic field. Evidence of pleural involvement is noted in the left costophrenic sinus.

Diagnosis: Marked mediastinitis, central low-grade bronchopneumonic infiltration. Thoracentesis was done and 1320 cc. of a slightly hazy straw-colored fluid was removed and sent to the laboratory for differential cell count, for presence of mitotic division figures and for inoculation in guinea pig. The report is as follows: "Sediment contains numerous mononuclears, moderate poly-

morphonuclear leucocytes and lymphocytes. No tumor cells recognized. No mitotic division figures found. Color, orange; translucence, moderate; consistency, fluid; butyric acid, plus four; white cells per c. mm. 1270; polymorphonuclear leucocytes 11 per cent; lymphocytes 89 per cent." All subsequent reports on the results of guinea pig inoculation were negative for tuberculosis.

A consultation with an eminent clinician was held at this time who made a diagnosis of Hodgkin's disease. It may be stated here that those of us who were most intimately connected with this case had at the very beginning considered the possibility of Hodgkin's disease but had ruled it out for want of clinical evidence, a finding which was subsequently confirmed by study of the tissue removed at the first biopsy. However in deference to this opinion, and because we were not fully convinced that either the clinical or pathological diagnoses were correct another biopsy was ordered. Because of conflicting interpretations in previous pathological studies we sent tissue from this biopsy to several laboratories where different pathologists rendered the following opinions.

First Laboratory Report: "Microscopic sections show no recognizable lymph gland structure. We find instead a diffuse growth of reticular cells with small lymphocytes scattered throughout but not in great numbers. The reticular cells vary from round to polyhedral in shape and vary markedly in size, many of them being very large, and the general picture is that of homogeneous sheets of cells. It would seem that the lymphocytes play a passive rôle in the formation of the tumor. It is usual in this particular group of tumors that when the reticular cells become so numerous the lymphocytes largely disappear. The neoplastic activity is not circumscribed but extends outward into the surrounding fibrous tissue and fat. Mitotic figures are occasionally found but not in all oil immersion fields.

Diagnosis: Thymoma. (Fig. 1.)

A consultation was held over this study with Dr. Ewing who concurred in the diagnosis made by our pathologist. His decision was accepted as final.

Second Laboratory Report: Microscopic sections show the normal architecture of the lymph node replaced by a diffuse lymphoid cell infiltration with some increase in the fibrous tissue comprising the stroma. Scattered eosinophiles are also found in the tissue. Histologically the tissue indicates a chronic (granulomatous) inflammation, not induced by the tubercle bacillus and probably not luetic. The presence of eosinophiles without the special cells characteristic of Hodgkin's disease precludes fairly definitely the possibility of this disease.

Diagnosis: Chronic (granulomatous) inflammation.

October 21, 1927: Patient is still running a sustained temperature of 100° to 102° F with little change in the swelling of the neck and cough or in her general condition.

October 22: Following the last biopsy report it was decided to treat the condition with radium. Accordingly 10 radium seeds were implanted in the mass in the left cervical region.

October 25: Patient was transferred at the request of the family to a sanitarium where she died on November 20, 1927.

Report of Autopsy

Body: Is that of an extremely emaciated white female aged 24, 163 cm. long. Abdomen scaphoid. General rigor and dependent livor mortis. Skin everywhere is covered with numerous discrete dry eruptions, 1 to 2 mm. wide, similar in appearance to old dried small scratch marks, the skin immediately adjacent appearing normal. On the mucocutaneous junction of left lower lip there is an irregular excoriation, 10 by 12 mm. Three old vertical linear scars are present in the left neck, 3, 3 and 2 cm. long. There is a small, dry, shallow decubitus ulcer over the left sacrum. In lower abdomen, just above both Poupart's ligaments and parallel to them are bluish ecchymotic areas, those on the right being more marked and larger, measuring 11.5 by 4 cm. Both supra- and infraclavicular fossae and axillary spaces contain numerous hard, discrete nodules, the largest being about the size of lima beans. In the subcutaneous tissues of left lateral thoracic wall in anterior axillary line is another indurated, freely movable nodule, 1.5 cm. in diameter. Just to the right of the sternum in the second intercostal space is a smooth, hard elevation, measuring 4.5 by 3.5 cm.

Both breasts are small, soft, and contain indefinite masses of soft mammary tissue. Pupils are regular, equal, moderately dilated, 6 mm. in diameter. There is a bilateral pterygium on nasal side of each eye. Teeth are good. Mucous membrane pale.

Incision: Y-shaped from midsternum to symphysis pubis. Panniculus adiposis practically absent. Muscles flabby and pale red.

Neck: Numerous hard superficial and deep cervical lymph nodes are present, the largest measuring 2 by 1 by 1 cm. In the anterior plane just beneath the very thin dark yellow subcutaneous fat is a very firm, fibrous opaquely white mass, 6 by 3 by 1.5 cm., with a tongue-like lower border fitting into the suprasternal notch. Thyroid is compressed into a thin colloid-containing mass. A lateral lobe measures 2.5 by 1.2 cm.

Thorax: (Fig. 2.) Left pleural cavity is almost completely obliterated, except for several locculi, about 5 cm. in diameter, containing turbid dark yellow fluid. In right pleural cavity there are about 500 cc. of blood-tinged fluid and a few firm fibrous adhesions posteriorly. Both parietal and visceral pleurae of the lateral wall and diaphragmatic surfaces are covered with a dirty gray fibrinous exudate. Visceral pleura of lower right lobe, especially the diaphragmatic portion, is greatly thickened and pearly white (Fig. 3). Parenchyma of right and left lungs are dark red and very edematous. Upper right lobe posteriorly, neár the hilum, contains a metastatic nodule 2 by 1.5 cm. in diameter, hard, very cellular. Anteriorly there is another, beneath pleura, 5 mm. in diameter. In left hilum are several similar nodules between the bronchial ramifications. Left apex is infiltrated with cellular, opaquely gray tumor tissue. There is a protuberant indurated growth, left paramedian, on the upper surface of the diaphragm, 5 by 3 cm., cut surface showing fibrous and more grayish cellular areas.

The external nodule in the right second interspace corresponds to a similar growth of equal size interiorly (Fig. 2) and merges with the mediastinal mass which pushes the heart over to the left. Pericardial sac contains about 150 cc. of blood-tinged fluid. Whole sac (Fig. 4) is lined by a shaggy, villous, brownish, fibrinous membrane, easily pulled off. Sac after formalin fixation measures 10 by 10 by 12 cm. *Heart* is in systole, 235 gm., 8.2 by 10 by 5 cm. All the coronary arteries are covered with tumor growth, the posterior branch measuring 9 mm. in its outer diameter. Aorta shows numerous small yellow raised atheromatous patches. Right ventricular wall, 0.5 cm., left 2 cm. Mitral valve shows many old vegetations. Pericardium in region of coronaries is as much as 5 mm. thick, firm and opaquely white.

Anterior mediastinal space is completely replaced by tumor tissue from superior border of sternum down to the diaphragm, to a depth of 4 cm. Superior thoracic aperture is completely infiltrated with opaquely white, hard tumor, completely surrounding trachea and esophagus. In fact the whole mediastinum down to esophagus in the midthoracic region consists of this firm opaque tumor tissue.

Abdomen: No free fluid present in peritoneal cavity. Large intestines are but slightly distended; several pultaceous small fecal masses are present in the terminal ileum. Stomach contains about

400 cc. of green, sour smelling fluid and some air. Gastric mucosa throughout shows quite a number of split-pea-sized grayish elevations, the apices of which contain a shallow ulcer about 3 mm. in diameter. Many perigastric glands are enlarged, resilient, the largest measuring 3 by 1.7 by 1.5 cm. Mesentery of ileum shows many minute to pea-sized calcified nodes. Intestinal mucosa is deeply injected and covered with blood-tinged mucus.

Liver: Measures 17 by 22 by 8 cm., firm and yellowish brown in color. No metastatic infiltration is present grossly on section. *Gallbladder* measures 5 by 2 cm., wall thin. Contents, about 5 cc. of dark green bile. *Spleen*, 8 by 5.5 by 2.5 cm., is soft, dark red and very pulpy. No tumor nodules found. *Pancreas* is firm and grayish yellow.

Left kidney, 285 gm., 13 by 8 by 6 cm.; *right* 275 gm., 13.5 by 7.5 by 5.5 cm. Their surfaces are coarsely lobulated. Capsule strips easily, exposing a mottled grayish red, smooth, swollen surface (Fig. 5). On section a similar mottled variegated dark red and opaquely gray appearance is noted. The medulla, which grossly seems to be uninvolved by the tumor infiltration appears as streaks of dark red radially towards the renal papilla. *Ureters* are grossly negative; the right passes from the left side, paramedian, over the bodies of the vertebrae towards the right brim of the pelvis. Right kidney here is obliquely situated, its lower pole lying in the left abdomen. *Bladder* is contracted, containing a few centimeters of slightly turbid urine. Mucosa is pale and very much rugose. *Adrenals* show no gross findings.

Uterus: Measures 6 by 5.5 by 2.5 cm., wall at fundus being 1.2 cm. thick. Mucosa is thin and pale. *Right ovary*, 4.5 by 2 by 1.5 cm., and left 5 by 2 by 1 cm., containing corpora lutea and albicantes. Tubal ostia are patent. Serosa of uterus and adnexae are deep purplish red in color.

Anatomical Diagnoses

Thymoma with extensions and metastases to lungs, peribronchial, cervical and axillary lymph nodes, stomach, kidneys and retroperitoneal and gastric lymph nodes. Acute fibrinous pericarditis (cor villosum) with effusion. Edema and congestion of lungs; chronic adhesive pleuritis (bilateral); hydrothorax (bilateral); acute fibrinous pleuritis (right). Healed tuberculosis of the mesenteric lymph nodes.

Microscopic Findings

Neck: Lymph nodes from neck show a diffuse necrosis, appearing as shadows of previous cells which refuse to stain differentially. However, there still remain nests of viable-looking tumor cells of the reticulum cell type of thymoma (Fig. 1), large polyhedral cells with large vesicular nuclei, distinct nucleoli and acidophile cytoplasm. Mitotic figures are numerous. The general picture of thrombosis, necrosis and fibrosis may be interpreted as a radiation effect on the tissues.

Mediastinum: Sections from the main tumor mass in the anterior mediastinum, pulmonary nodules, diaphragmatic and enlarged axillary nodes all present a similar picture of diffuse tumor cell overgrowth, made up of large polygonal cells with vesicular nuclei, distinct nucleoli and acidophile cytoplasm. Numerous mitotic figures may be seen. Similar tumor cells invade the peri-esophageal and peritracheal tissues. Vessels here contain fibrin thrombi. Alveoli of lungs near area of tumor infiltration contain dense fibrinous exudate, coagulated serum or red blood cells. Vessels in this region are engorged with blood or plugged with thrombi. Areas of necrosis are present. Beneath the fibrinous exudate the subpleural tissues are infiltrated with tumor cells.

Heart: Beneath the dense fibrinous exudate on the visceral and parietal pericardium is present a similar tumor infiltration as seen under the pleura (Fig. 6). Myocardium itself is not involved.

Gastro-Intestinal Tract: Gastric and intestinal mucosae show marked postmortem decomposition, are injected and infiltrated with numerous round cells. The lymphocytes of a large perigastric lymph node are completely replaced by tumor elements. Submucosal vessels are deeply engorged. In sections from the nodules of the gastric mucosa tumor cells and mitotic figures are plentiful (Fig. 7).

Liver, Pancreas and Spleen: Essentially negative.

Kidneys and Adrenals: A widespread tumor replacement of renal tissue leaves very little cortical parenchyma here and there (Fig. 8). In the more involved areas the glomeruli can be seen as single entities, lying in a bed of congested tumor tissue. Adrenals are negative except for minute focal petechiae in both cortex and medulla.

Uterus and Adnexae: Show a general congestion. One section of the ovary shows metastatic tumor areas with many mitotic figures.

Final Diagnoses

Thymoma with extension to pleura, lungs, pericardium, diaphragm, and metastases to cervical, peribronchial and axillary lymph nodes, stomach, perigastric lymph nodes, kidneys, ovary, and invasion of sternum. Acute fibrinous pleurisy associated with tumor cells. Hydrothorax. Acute fibrinous pericarditis with effusion associated with tumor cells. Edema and congestion of lungs. Chronic adhesive pleuritis (bilateral). Healed tuberculosis of mesenteric lymph nodes. Chronic passive congestion of liver and spleen.

Comment

This case of malignant thymoma presents many points of clinical and pathological interest, the outstanding features of which are: (*a*) the difficulties in reaching a definite conclusion as to the true nature of the process involved; (*b*) the apparently slow growth of the neoplasm during the early months of progress; (*c*) the absence of any but relatively trivial clinical symptoms; and (*d*) the extensive metastases to distant regions without definite disturbances in function of the organs involved.

Attention has already been called to the confusion caused by early pathological, serological and roentgenological studies. The biopsy findings show the importance of obtaining a biopsy before the therapeutic use of the roentgen ray. Our reliance upon the results of the Vernes' flocculation test for tuberculosis has received some justification in a recent contribution by Baylis,[13] whose observations in a series of 250 cases explains our error in interpreting the figures recorded. It appears that positive results are obtained in the presence of pulmonary newgrowths of non-tubercular origin which have undergone necrosis, and are therefore unreliable as an indication of active tuberculosis. Clinically the latter condition was ruled out at the first exmination, for there were no physical signs and no subjective complaints suggestive of a Koch infection. The substernal and subclavicular dullness was continuous with the mass in the neck and was not attributed to pulmonary consolidation. At this stage of the disease there were no sharply outlined enlarged glands as seen in tuberculosis; on the contrary the left cervical mass consisted of a single diffuse, goiter-like tumor. It was not until a microscopical examination of gland tissue showed changes suggestive of tuber-

culosis together with a positive Vernes' test with the subsequent development of pleurisy, that our attention was again attracted to the possibility of a tubercular process.

A diagnosis of syphilis was entertained because of the persistent ulceration of the pharynx of unknown origin which resisted ordinary medication. Repeated Wassermanns however were negative. Later when a histological study of the tissue removed at biopsy suggested a syphilitic lesion, a provocative Wassermann and a Vernes' flocculation test for lues were done, but again reported negative. Lymphosarcoma and Hodgkin's disease had been given due consideration but the appearance of the neck was not typical of these conditions and the first biopsy showed none of the characteristic histological changes ascribable to them.

The apparently slow growth during the early stages of our case followed by a rapid progress in the last two months is in accordance with the description of similar cases of thymoma reported by other observers. Apparently the process grew so insidiously that the structures of the thorax gradually accommodated themselves to the invading mass without producing marked pressure symptoms. Considering the size of the neoplasm removed at autopsy it is astonishing that the patient presented so few symptoms during life. The duration of our case from the time when the patient first consulted us until death was a little short of four months, during the first two months of that period the patient's chief concern was the enlargement of the neck and the disfiguring discoloration surrounding the eyes. She complained of a slight cough, soreness of the neck, dysphagia, nervousness, insomnia and loss of appetite, but all these disturbances disappeared on occasions and she would then enjoy intervals of perfect comfort. It was surprising how well she felt considering the seriousness of her condition and we were impressed with the patient's cheerfulness and state of optimism, a mental attitude comparable to that so often attributed to subjects of tuberculosis. Not until the lung parenchyma became invaded, about seven weeks prior to death, did she suffer real discomfort. She then complained of an annoying cough with considerable pain in the right chest. It was also surprising how little disturbance was occasioned by the metastases to distant organs. The kidney parenchyma showed widespread replacement by tumor elements yet the last urinalysis was negative except that only 400 cc. were excreted during twenty-four

hours. Unfortunately a blood chemistry was not done to determine whether nitrogen retention was present.

Attention is also directed to the manner of invasion of the lungs. Previous observers reported the involvement of lung tissue by direct extension whereas in our case this was followed both by metastasis and extension. The extension over the visceral and parietal pericardium was by continuity from the primary tumor mass. The peculiar pinkish color phenomenon surrounding the eyes remains unexplained and several dermatologists who had been consulted were also unable to offer a satisfactory explanation.

In reference to the treatment it is worthy to note that a single exposure to roentgen-rays was followed by a shrinking of the substernal mass although no effect upon the cervical tumor was demonstrated. The patient's condition progressed so rapidly to a fatal issue that it was impossible to draw any conclusions as to the action of the radium seed. The tumor cells had already entered the circulation when we were first consulted as evidenced by the metastasis to the cervical glands and it is therefore extremely doubtful that X-rays or radium instituted at that time would have checked the condition. The most we could have hoped for would have been an amelioration of symptoms and a temporary remission in the progress of the growth.

No authentic cases of cures have been recorded in malignant thymoma. Groover, Christie, Merritt and Coe [14] reported a marked remission in the tumor in one case following deep radiation and an apparently complete and permanent disappearance in another but the nature of the tumor was not proved pathologically.

In reporting the data on this case of thymoma it is realized that no method of reasoning is offered whereby a correct diagnosis of this malignant neoplasm can be made clinically, for the ultimate decision will always rest with the pathologist. It is believed however that the publication of our experiences will in the occurrence of mediastinal neoplasms stimulate a closer search of the thymus gland as the possible origin of malignancy and thus lead to the recognition of such a tumor at a stage early in development when surgery or radiation may effect a cure.

Summary

The pathological studies on this case were made by Dr. Louise H. Meeker,[15] who summarizes the findings as follows:

All studies of thymoma end more or less in theoretical discussions with quotations from the authoritative works of Hammar,[16] Schridde,[17] Danchakoff [18] and Maximow.[19] The opposed views of these writers as to the genesis of the thymic cells accounts for the persistence of the indefinite term "thymoma." The rarity of "thymoma" accounts in part for our inadequate knowledge. According to our files the first record of thymoma in our hospital was in June 1926.

In this case we have a malignant tumor originating in the thymic region as evidenced by the clinical history and substantiated by the anatomical findings.

Grossly the tumor is white and leathery and has no sharp boundaries. It fills the entire mediastinum extending upward about the larynx, displacing the thyroid, ensheathing the large vessels of the neck and invading the cervical glands. Its lateral and downward extensions are directly along the pleura and pericardium following the vena cava and prevertebral connective tissue below the diaphragm and spreading to the viscera of the abdomen and pelvis. The abdomen and pelvic involvements include both kidneys, the stomach (intestine?) and ovary. Extension below the diaphragm is rare and no single previous thymoma reported has equalled the widespread involvement that has occurred in this case.

The striking features of the finer detail are first, the evident extension by way of the lymphatics and second, the pronounced infiltrating character of the neoplastic tissue especially shown by the obliteration of structural landmarks in the organs invaded. The radiated portions of the growth in particular show many areas of necrosis.

Microscopically the cells forming the tumor are of one general type growing diffusely throughout a scanty stroma although in some areas the stroma may be abundant. They are small cells for the most part with vesicular nuclei having one nucleolus and with scanty slightly basophilic or acidophilic cytoplasm. Closely intermingled with these just named cells there are large cells also with vesicular nuclei having nucleoli and more abundant and paler cyto-

plasm often with one to several processes. Many of the cells contain acidophile granules, still others are phagocytic and mitotic figures are occasionally fairly numerous and pyknotic forms are common. Irregular calcific deposits found only in sections from the true thymic region are interpreted as degenerated Hassal's corpuscles. We have interpreted the tumor cells as pretty closely resembling so-called small thymic cell and especially the thymic reticular elements. Hassal's corpuscles are not a part of the newgrowth.

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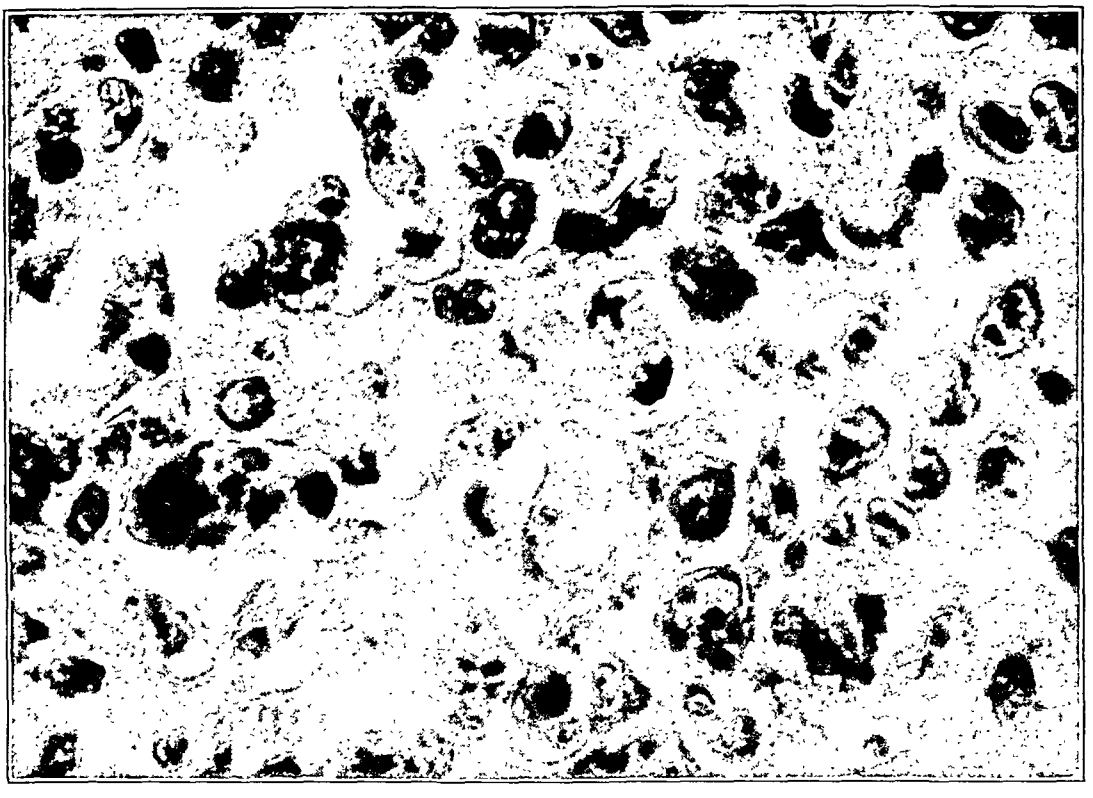
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DESCRIPTION OF PLATES

PLATE 10

FIG. 1. High power. The polymorphous reticular type cells are well shown.

FIG. 2. Photograph of gross specimen showing the masses attached to the sternum and the diffuse growth filling the mediastinum and extending over the pleura, pericardium and diaphragm.



1



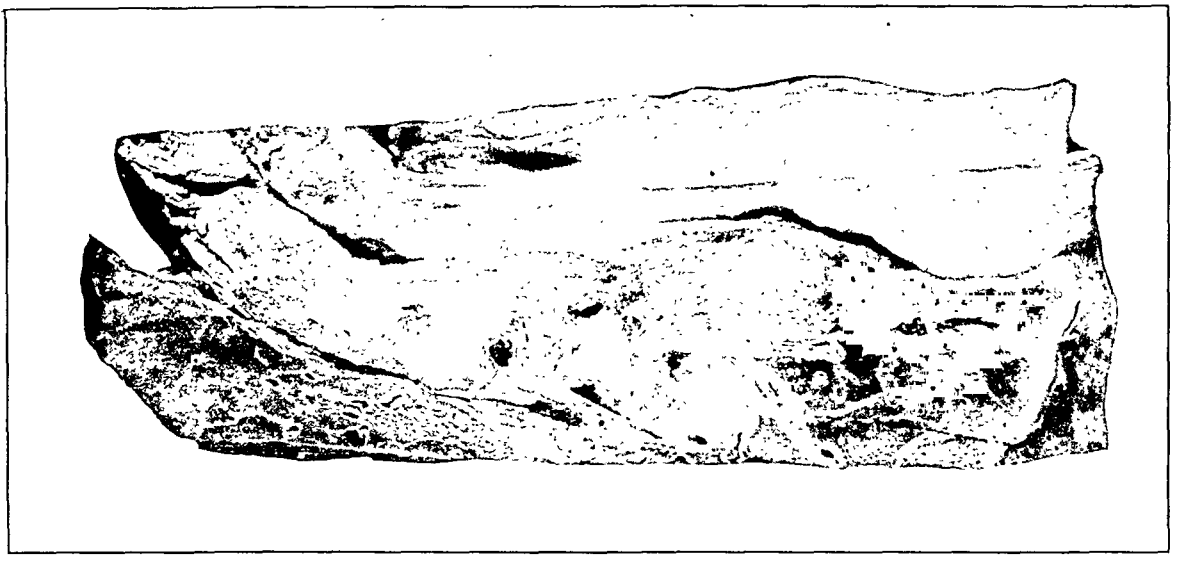
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Herriman and Rahte Malignant Thymoma with Metastases

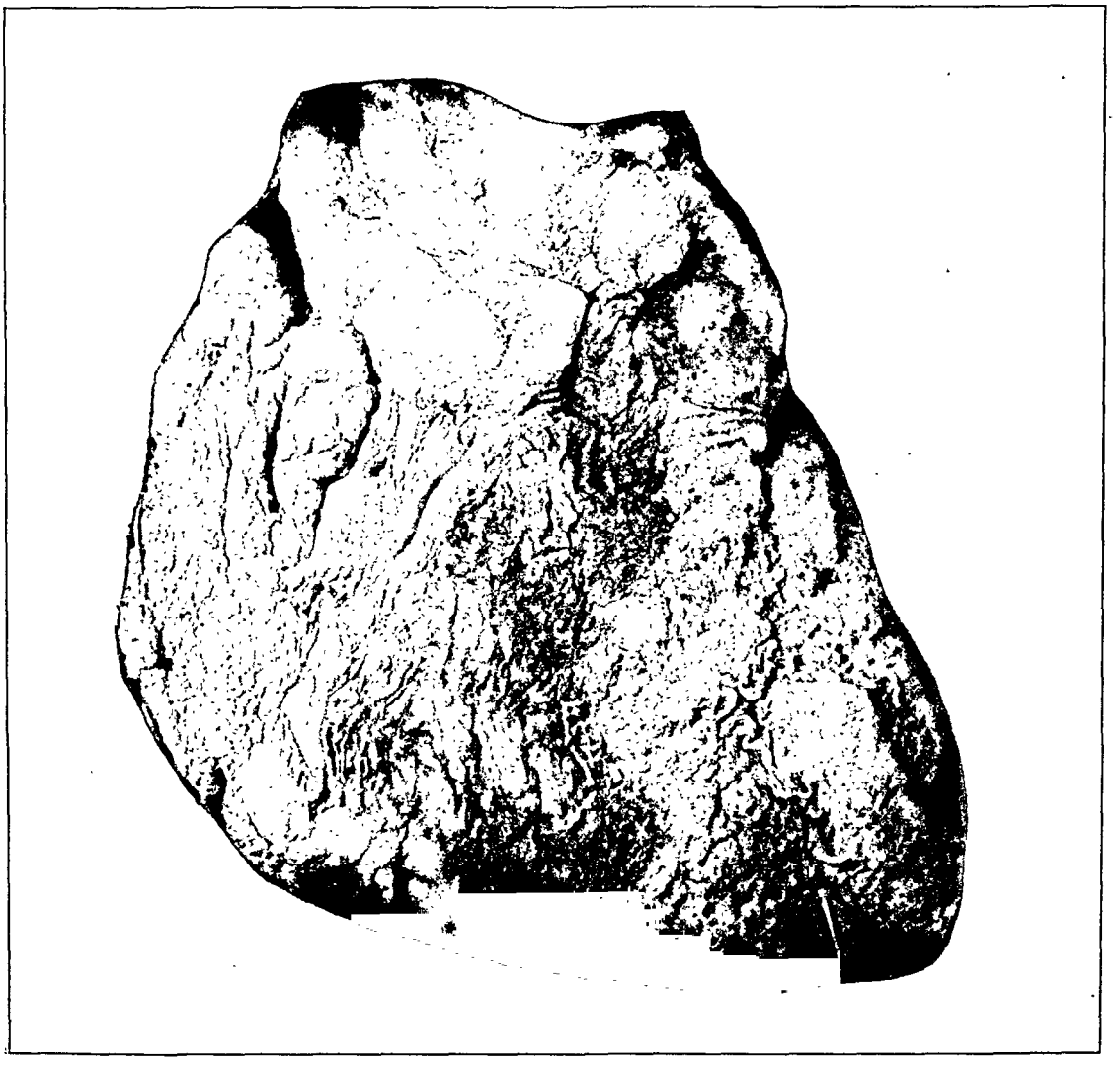
PLATE II

FIG. 3. Showing the pleural thickening by the tumor growth.

FIG. 4. Photograph of gross specimen. The entire heart is covered with a shaggy growth of tumor cells.



3

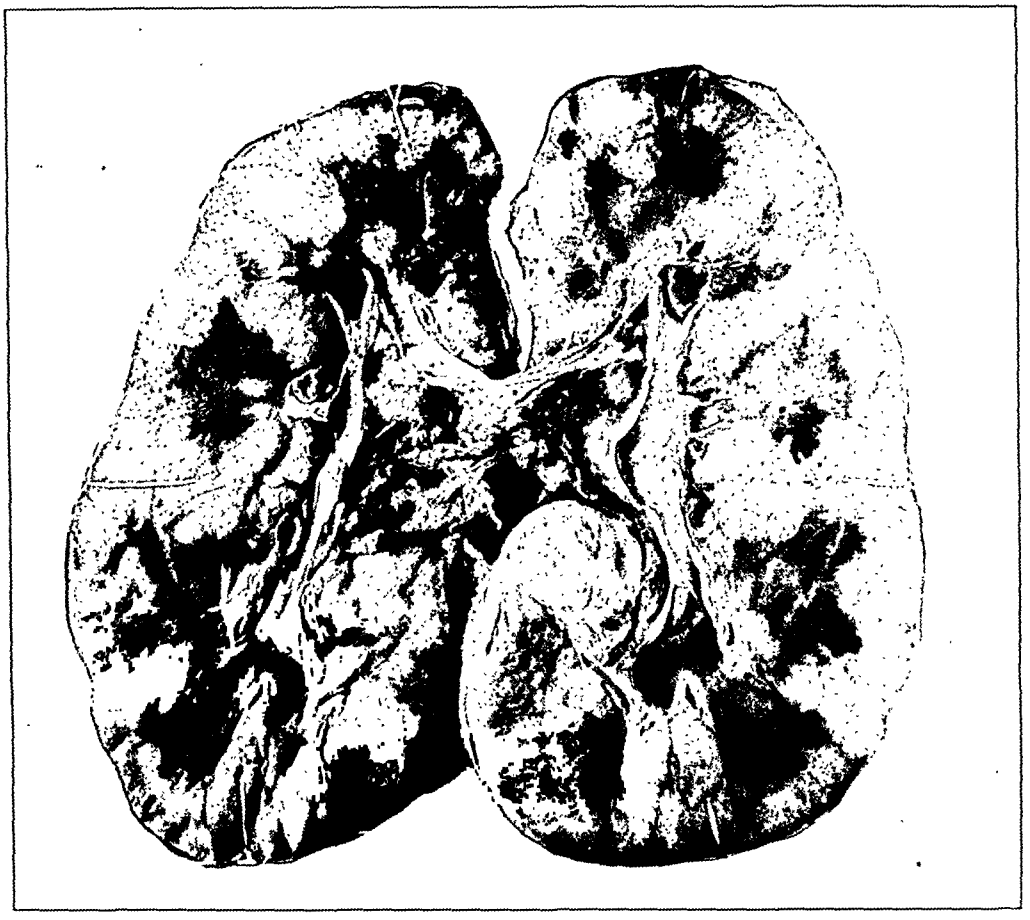


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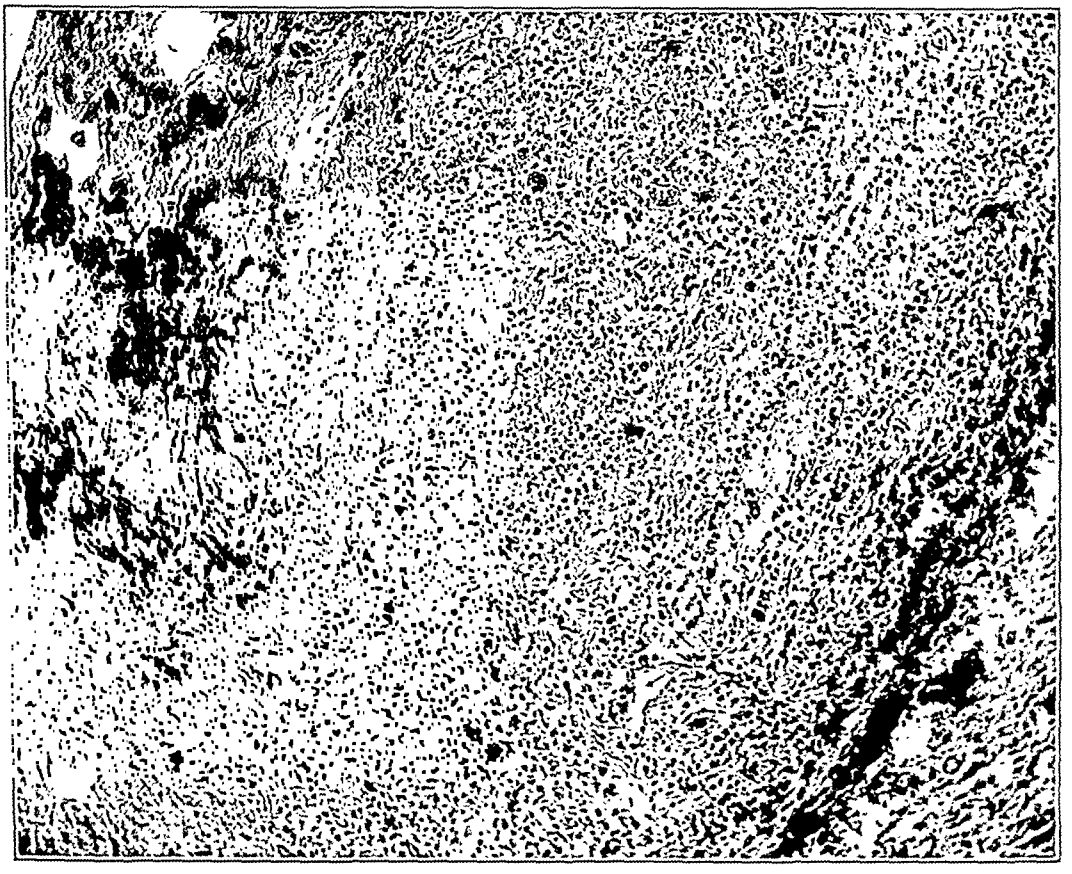
PLATE 12

FIG. 5. Photograph of kidney. All the pale areas are formed of tumor metastases.

FIG. 6. Photomicrograph of pericardium showing the thick mantle of tumor cells.



5

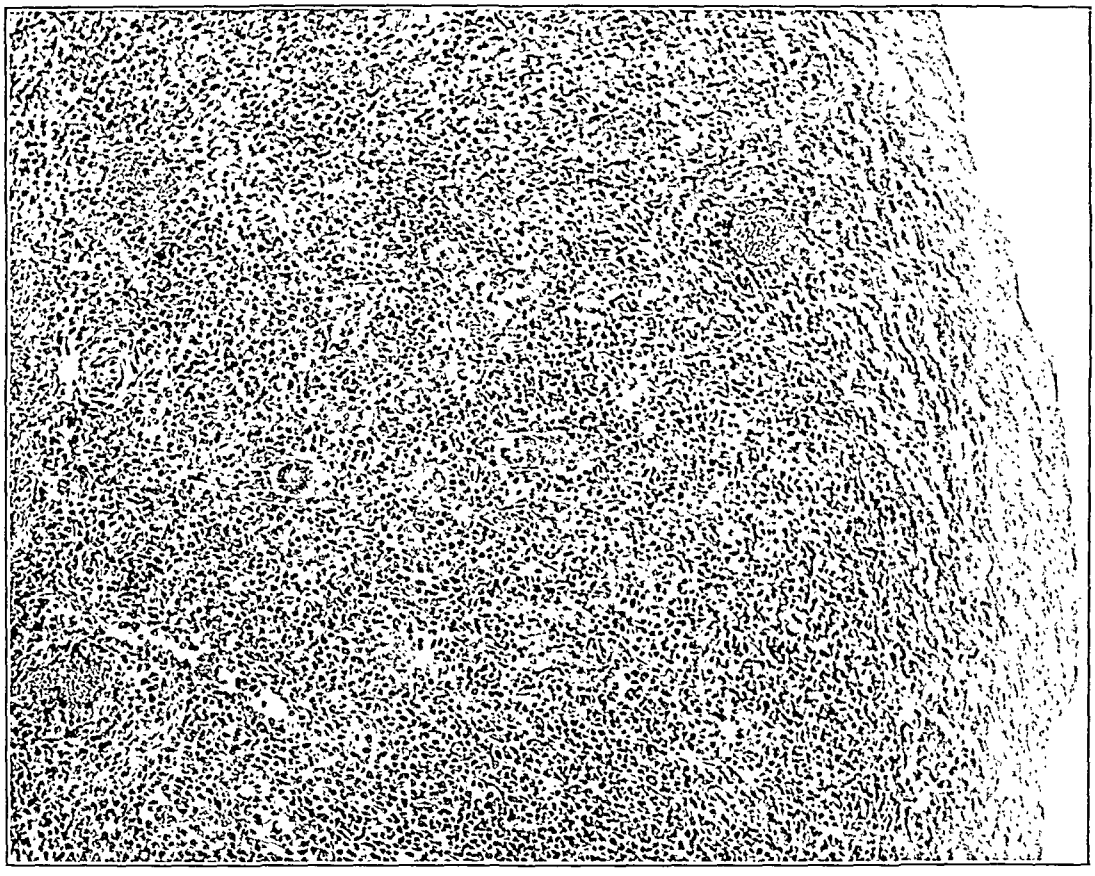


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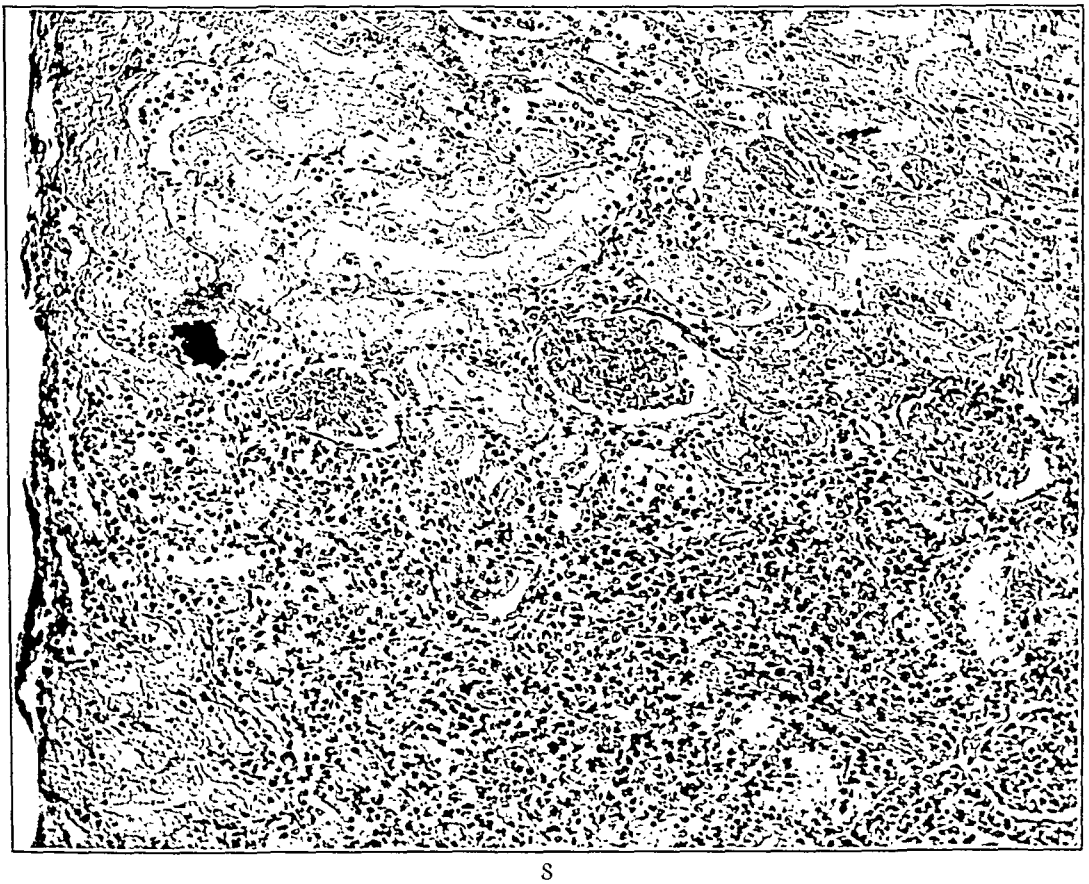
PLATE 13

FIG. 7. Photomicrograph of nodule in the mucous membrane of the stomach.

FIG. 8. Photomicrograph showing the invasion of the kidney by tumor cells.



7



8

Herriman and Rahte Malignant Thymoma with Metastases

THE INTIMAL LESION OF THE AORTA IN RHEUMATIC INFECTIONS *

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Rheumatic aortitis is now a well recognized entity and several instances have appeared in the literature. Although the first recorded observations on rheumatic arteritis date back to Bouillaud[1] in 1840, it was not until the work of Klotz [2] and Rabé [3] that accurate pathological data were collected on the subject. Klotz pointed out the flame-like scars about the nutrient vessels in the media and the cellular infiltrations of the adventitia. It is, however, to Pappenheimer and VonGlahn that we owe a complete histopathological description of the vascular lesions in rheumatic fever both in the aorta and the smaller vessels.[4, 5] In their first paper on the subject of aortitis in this disease [5] they found no intimal lesion but noted in addition to Aschoff bodies in the adventitia, dense and acellular scars in the media about the nutrient vessels. Here the initial lesion was described as a swelling, fragmentation, and lysis of the collagen fibrils. They subsequently [6] reported in a child 9 years of age with progressive recurrent rheumatic fever an instance of rheumatic aortitis with previously undescribed cellular infiltrations of the media as well as fairly typical changes in the adventitia. In the outer two-thirds of the media the vessels were thickened and about these there were polymorphonuclear leukocytes, lymphocytes, and larger elements having the structural and staining characteristics of "Aschoff cells."

More recently, the same authors reported two further instances of cellular rheumatic lesions in the aorta.[7] In one the intima was involved microscopically. In the other, accompanying typical changes in the endocardium of the left auricle and in the mesenteric, renal, and coeliac vessels, glistening brownish translucent patches of ridges were noted in the intima of each sinus of Valsalva and of the ascending aorta, resembling in many respects the endocardial lesions of the auricle. Microscopically the lesions were predomi-

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nately in the intima and the subjacent portions of the media. Bands of non-nucleated fibrillar tissue were found in the intima bordered by rows of deeply staining cells with basophilic cytoplasm and one or occasionally two large vesicular nuclei containing a central dense clump of chromatin. The intimal endothelium appeared swollen and polygonal and gave the appearance of migrating into the subjacent stroma. Occasional clefts were noted in the intima which in places appeared to communicate with the lumen of the aorta and were filled with red blood cells and bordered by polyhedral cells. No cholesterin crystals were found. The media showed a complete loss of muscle fibers over large areas with only collagen and elastic fibers persisting. In places the elastic coat was thinned out and ruptured. In the outer two-thirds of the media were seen perivascular infiltrations of the type previously described.*

These are not the only instances of intimal lesions of the aorta in rheumatism reported in the literature, although the few previously described cases are not unequivocal.** We are reporting two further instances of gross rheumatic involvement of the intima of the aorta.

CASE 1. *Clinical History:* No. 15817, M. P., male, age 10½ years, entered Montefiore Hospital on Feb. 5, 1928, with the complaint of palpitation and shortness of breath on exertion. During the preceding five years he had had recurrent attacks of rheumatic fever with evidence of cardiac involvement. On examination at the time of admission he presented the physical signs of mitral stenosis and insufficiency and aortic insufficiency. His blood picture was normal. During the greater part of his stay in the hospital he was febrile and developed a marked anemia. A few days before exitus he complained of severe precordial pain and a loud friction rub was heard over the apex and lower edge of sternum. The child died suddenly seven weeks after admission.

The autospy No. (4261) was performed 5½ hours after death.

The anatomical findings were: recurrent rheumatic endocarditis, mitral and aortic valves, and left auricle; mitral and aortic insuf-

* Kugel and Epstein [8] have recently reported microscopic rheumatic lesions of the pulmonary artery resembling the aortic lesions described by Pappenheimer and Von Glahn. They noted the frequency of involvement of the pulmonary musculo-arterial junction.

** A complete review of the literature bearing on lesions of the blood vessels in rheumatic fever may be found in the publications of Pappenheimer and VonGlahn. Chiari [9] has recently described six instances of chronic rheumatic infection with adventitial lesions of the aorta and its main branches. These consisted of perivascular infiltrations with "Aschoff cells," plasma cells and lymphocytes.

ficiency; cardiac hypertrophy and dilatation; rheumatic aortitis; organizing serofibrinous pericarditis; partial atelectasis left lung; chronic passive congestion abdominal organs; ascites.

Heart: The heart weighed 600 gm. It was covered by a shaggy exudate. The mitral and aortic valves presented the typical changes of recurrent rheumatic valvulitis. In addition to an extremely rich vascularization of the aortic leaflet of the inflamed mitral valve, there were two other interesting findings. One was the presence in the endocardium of the left ventricle, about 0.5 cm. below the right anterior aortic cusp, of an oval slightly raised patch about 1.5 cm. in diameter and 1 to 2 mm. in thickness, consisting of soft, yellowish gray tissue with a finely striated surface. It was well demarcated from the surrounding endocardium by a serrated margin. There was a similar plaque, 0.5 cm. in diameter, on the ventricular surface of the aortic leaflet of the mitral valve about 8 mm. below the junction of the posterior and left anterior aortic cusps. The other interesting finding was the presence of several transverse ridges in the thickened endocardium of the dilated and hypertrophied left auricle.

Aorta: The aorta (Fig. 1) on its intimal surface about 0.5 cm. above the commissural junction of the posterior and right anterior cusps presented a well demarcated oval plaque about 1 by 0.7 cm. in size and raised about 1 mm. Grossly it involved only the intima. The surface had a pale brownish yellow color, and in the central portion well defined longitudinal furrows were seen. About 0.5 cm. above the commissure between the posterior and left anterior cusp was a similar plaque about 7 mm. in diameter presenting an irregular, markedly injected, shallow central depression. A third plaque of this nature, 4 mm. in diameter, about 0.5 cm. above the margin of the left anterior sinus of Valsalva, was removed for microscopic study. There were a few simple atheromata of the intima in the ascending and lower abdominal portions of the aorta, extending for a short distance into the common iliacs. The other large arteries and the coronary arteries were grossly normal.

Microscopical Findings

Left Auricle: In a section taken just above the mitral valve the endocardium is markedly thickened. Just beneath the endothelium is an accumulation of fibrin. Beneath this the tissue is infiltrated with numerous round cells and elongated, deeply basophilic connec-

tive tissue cells containing spindle-shaped nuclei, and arranged in horizontal orientation to the surface. Sections through the thickened ridges noted in the gross show a marked cellular infiltration. In part this consists of lymphocytes and peculiar comma-shaped cells arranged in places in a vertical orientation to the surface on either side of bands of swollen collagen fibers. In the depths of the endocardium is a diffuse infiltration with polymorphonuclear leukocytes and lymphocytes, continuous with a similar exudate in the subjacent myocardium, in places following the course of blood vessels. The myocardium itself shows a moderate hypertrophy of the muscle fibers and a diffuse fibrosis, chiefly perivascular. In this connective tissue are seen a few round cells and an occasional "Aschoff cell."

Left Ventricle: The endocardium is thickened and infiltrated with numerous round cells and epithelioid cells with basophilic cytoplasm and large vesicular nuclei. These appear very much like "Aschoff cells." Other sections through the ventricular wall show an organizing exudate in the thickened pericardium with accumulations of round cells, leukocytes, and fibroblasts, some with basophilic cytoplasm and large vesicular nuclei. In the myocardium are perivascular accumulations of lymphocytes, plasma cells and occasional polymorphonuclear leukocytes and large "Aschoff cells."

Pulmonary Artery: (musculo-arterial junction). The artery itself shows no lesions of the wall, but at the myocardial junction there are perivascular accumulations of round cells, polymorphonuclear leukocytes and "Aschoff cells."

Coronary Artery: A section shows only a low-grade atheromatosis.

Aorta

A section through the plaque (Figs. 2, 3, 5, 6) shows a striking picture. There is a loss of the lining endothelium and the intima is replaced by a broad layer of fibrin. Beneath this layer is a zone of connective tissue rich in large dilated capillaries and containing numerous cells arranged for the most part with their long axes perpendicular to the surface, in places grouped about capillaries. They are large, irregular, spindle-shaped, connective tissue cells and in one area invade the fibrin zone, penetrating to the free surface of the intima. The greater portion of the exudate in its outer third contains numerous small pyknotic distorted nuclei, the exact nature of which cannot be determined. Some are elongated and arranged

vertical to the surface. At the periphery of the plaque the fibroblasts have reached the surface of the exudate forming a closely packed layer in which the long axes of the cells are arranged parallel to the surface. In addition there are in this area a number of peculiar irregular, elongated, spindle-shaped cells with large, irregular, oval, sometimes slightly lobulated, vesicular nuclei, some of which show pale basophilic spherules as well as occasionally a prominent nucleolus. The cytoplasm is deeply basophilic and indistinctly granular. In some sections through the organized portions of the exudate there are deep and irregular narrow endothelial-lined clefts communicating with the lumen of the aorta. The intima of the adjacent aorta where no fibrin exudate is present is very cellular, containing numerous closely packed cells of the type just described. These cells were seen also in the pericardial organization tissue described above, and we have seen identical cells in the granulation tissue of a non-rheumatic organizing fibrinous pericarditis. Although they sometimes approximate the "Aschoff cells" in appearance they are most probably actively proliferating fibroblasts. A few lymphocytes and an occasional polymorphonuclear leukocyte are also seen. No plasma cells or giant epithelioid cells of Aschoff are present. With Weigert's elastic tissue stain it is seen that the fibrin exudate is almost entirely within the limits of the internal elastic membrane, which is fragmented and frayed out.

Media: In the inner portion of the media and at the site of the granulation tissue, the elastic fibers show extensive distortion, destruction, fragmentation and fraying. The nutrient vessels penetrate the outer third of the media and in an area corresponding to the extent of the intimal exudate they are surrounded by peculiarly radiating cells lying in a pale ground substance. These cells (Fig. 4) seem to sweep downward in the form of a fan toward the intimal surface and are most numerous along the wide flare of the "fan." The cells, although somewhat irregular in size, are uniformly spindle-shaped with dark staining large oval nuclei and deeply basophilic cytoplasm. There is an occasional polymorphonuclear leukocyte. The ground substance of the fan-like areas consists of a delicate, fibrillar, very pale staining connective tissue containing several small capillaries in addition to the main nutrient vessel. The elastic tissue in these areas shows extensive destruction, fragmentation, clumping, and fraying. A most striking feature is

that these fan-like areas are present only about the nutrient vessels corresponding to the area of intimal exudation. The remaining nutrient arteries show smaller areas of cellular infiltration with round cells, a few proliferating fibroblasts and leukocytes, and several groups of two or three round and oval wandering cells with homogeneously dark staining round nuclei and deeply basophilic non-granular cytoplasm.

Adventitia: Throughout the adventitia, frequently perivascular, are numerous small cellular infiltrations made up chiefly of lymphocytes, a few large wandering cells and an occasional Aschoff giant cell. The scattered, large, wandering cells have oval dark staining nuclei and show extreme variations in shape, many having long protoplasmic processes. Several have distinctly granular cytoplasm not unlike that of mast cells.

Thoracic and Abdominal Aorta: Sections from the thoracic and abdominal portions of the aorta show the characteristic mild perivascular infiltrations limited to the adventitia which have been described by Pappenheimer and VonGlahn.[4] They are made up chiefly of round cells, but there are also present a few plasma cells and in places a few leukocytes and an occasional mast cell. In several instances these infiltrations surround large multinucleated cells, typical of Aschoff giant cells.

Smaller Arteries: The smaller arteries in various portions of the body also show evidence of involvement. In one of the mesenteric arteries in an area just beneath the endothelium there are seen round cells and a few distorted cells with elongated nuclei, whose identity cannot be established. In the surrounding fat is an extensive infiltration of round cells and polymorphonuclear leukocytes. A few plasma cells are present, but no definite "Aschoff cells." Similar infiltrations are seen in the fat surrounding the small vessels throughout the section. In the hilum of the spleen about some of the small arteries there are small accumulations of round cells and an occasional leukocyte but no "Aschoff cells." In the section of kidney adjacent to the wall of a vein there is an accumulation of round cells and an occasional plasma cell. In the perisuprarenal fat adjacent to the small vessels are seen accumulations of leukocytes and several epithelioid and multinucleated giant cells, whose cytoplasm, however, takes only a faintly basophilic tint with the Pappenheim stain. No acute fibrinous lesions of the wall such as described

by Pappenheimer and VonGlahn [5] were noted. Pappenheim stains of sections of the aorta as well as the other organs failed to reveal the presence of bacteria.

CASE 2. *Clinical History:* No. 04369, A. L., female, aged 16 years, admitted to Montefiore Hospital on Nov. 18, 1920, with the complaint of dyspepsia, precordial distress, weakness, and pains in the joints for a period of about fourteen years. These symptoms began following an attack of scarlet fever at the age of three, and had since then recurred at irregular intervals. On examination the patient was moderately anemic and presented the physical signs of mitral and aortic stenosis and insufficiency with cardiac hypertrophy. The liver and spleen were palpable. There was no edema or ascites. During her stay in the hospital she developed an acute exacerbation of her rheumatic infection with cardiac involvement following an attack of tonsillitis. She recovered, however, and remained ambulatory and in fairly good condition for four months before exitus. She died suddenly during sleep, ten months after admission.

The autopsy (No. 3473) was performed twenty-eight hours postmortem by Dr. D. P. Seecof.

The anatomical findings in brief were: recurrent rheumatic endocarditis, mitral and aortic valves, left ventricle; mitral stenosis and insufficiency; aortic stenosis and insufficiency; rheumatic myocarditis; rheumatic aortitis; chronic adhesive pericarditis; healed mural thrombus, right auricle; chronic passive congestion of viscera.

Heart: The heart was markedly enlarged, the pericardial cavity obliterated by thin sheet-like fibrous adhesions. On section was found an advanced, in part calcified, in part ulcerated, rheumatic involvement of the mitral and aortic valves, as well as slight thickening of the pulmonic and tricuspid leaflets. The left auricle and ventricle were hypertrophied and dilated, the left ventricular wall measuring 20 mm. The musculature contained numerous pearly white scars. In the region of the interventricular septum, immediately below the aortic ring, the intima of the left ventricle was the site of an irregularly thickened, fibrous, flattened elevation, 2 cm. in diameter and 1 to 2 mm. in thickness.

Aorta: In the ascending portion of the aorta was an irregular, large, brownish yellow, flattened plaque similar in appearance to the one described in the left ventricle.

MICROSCOPICAL FINDINGS

Heart: The heart shows numerous perivascular scars which in places are infiltrated with round cells and multinucleated cells of

the Aschoff type. The muscle fibers are hypertrophied. At the site of the endocardial plaques in the left ventricle the endocardium is thickened and contains sparsely cellular, partly hyalinized, edematous connective tissue, in which no characteristic elements can be discerned.

Aorta

Immediately beneath the endothelium, the intima and adjacent inner portion of the media are irregularly thickened (Fig. 7) forming a prominent elevation which presents deep endothelial-lined clefts communicating with the lumen of the aorta. Just beneath the endothelium at the peripheral margin of the plaque are numerous spindle-shaped cells arranged parallel to the surface and having the appearance of connective tissue cells. In addition, there is a moderate number of round and oval cells with round and oval, homogeneously dark staining nuclei and strongly basophilic cytoplasm, as well as an occasional leukocyte. In the intima, limited by the internal elastic membrane, there is a peculiar pale staining, finely fibrillar tissue containing a few scattered capillaries and diffusely infiltrated with a moderate number of lymphocytes and many fibroblasts, some having more or less basophilic cytoplasm. The fibrillar material stains as fibrin with Mallory's phosphotungstic acid hematoxylin.

Media: In the portion of media comprising the deeper layers of the plaque are seen diffusely scattered connective tissue cells lying in small lacunae in a stroma of swollen fibrillar tissue, which in places is highly vascular. Just external to the area of greatest vascularization is a very dense infiltration with irregular, closely packed, oval and spindle-shaped connective tissue cells, some with basophilic cytoplasm, the long axes of which are arranged perpendicular to the intimal surface. Among these are scattered several large cells with intensely basophilic staining cytoplasm and multiple or lobulated dark staining and vesicular nuclei, "Aschoff cells." A few round cells with dark staining homogeneous nuclei and basophilic cytoplasm are present but no definite plasma cells. In the region of these dense cellular infiltrations the Pappenheim stain reveals numerous small particles of basophilic staining material. The elastic tissue of the internal elastic membrane is in places thinned out and fragmented. The elastic tissue of the media which is included in the plaque, particularly where the latter is thickest, is

extensively fragmented, frayed and distorted. There is a very striking loss of muscular elements in the remainder of the media at the site of the plaque. The nutrient vessels penetrate as deeply as the middle of the media and are accompanied by small perivascular accumulations of lymphocytes, occasional leukocytes, plasma cells and a scattering of mast cells. A few of the latter have nuclei characteristic of those of the plasma cells. In this outer, vascularized portion of the media are seen striking destructive changes in the elastic tissue in addition to a disappearance of the muscle fibers over a large area corresponding to the site of the intimal plaque.

Adventitia: The adventitia is highly vascular; the connective tissue is increased and has an edematous appearance. About many of the capillaries there are fairly dense cellular accumulations, consisting chiefly of lymphocytes and plasma cells. A few of the latter show transition forms to plasma mast cells. There are also a few "Aschoff cells" among these infiltrations.

Sections of the remaining organs show evidences of marked chronic passive congestion.

Discussion

In a consideration of the various types of lesions described in the aorta on rheumatic fever, three groups may be distinguished:

1. Involvement of the adventitia alone, with perivascular infiltration and the formation of Aschoff bodies (Fig. 4).

2. Involvement of the adventitia and the media. The lesion at the latter site may be in the form of (*a*) an acute, cellular, perivascular infiltration in the outer third with destructive changes in the media immediately around the vessels (Fig. 6); or (*b*) perivascular scars of longer standing at this site, the consequent interference with the blood supply resulting in a patchy loss of muscle cells in the remainder of the media (Fig. 4).

3. Involvement of all three layers of the aorta with either (*a*) an acute process, as in Case 1, in the form of a fibrinous intimal exudate accompanied by cellular infiltrations about the nutrient vessels in the outer third of the media with perivascular destructive change in this region; or (*b*) a lesion of longer standing as in Case 2, consisting of an organized intimal plaque accompanied by characteristic infiltrations about the nutrient vessels, with extensive muscular atrophy throughout the corresponding portions of the media.

In view of the strict gradation which can be discerned among these various types of lesions, we are inclined to believe that they represent rheumatic infections of the aorta by way of the vasa vasorum, the variations in the picture depending upon the severity of the infection and the chronicity of the lesion. We do not think that the intimal lesion is to be considered as a direct infection from the intimal surface.

Summary

Two instances of macroscopic involvement of the aorta in recurrent rheumatic fever are described. A striking feature, which we believe has not been previously described, is the presence in one of the cases of an acute fibrinous lesion of the intima. In brief, the characteristics of the lesion are:

1. Aschoff bodies in the adventitia.

2. Perivascular (in the acute stage, fan-like) infiltrations in the outer third of the media, with destruction of elastic tissue and muscle elements.

3. Recent and organized fibrinous plaques in the intima, the connective tissue cells comprising the vascular organization tissue having a characteristic vertical orientation at the base of the intimal lesions.

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DESCRIPTION OF PLATES

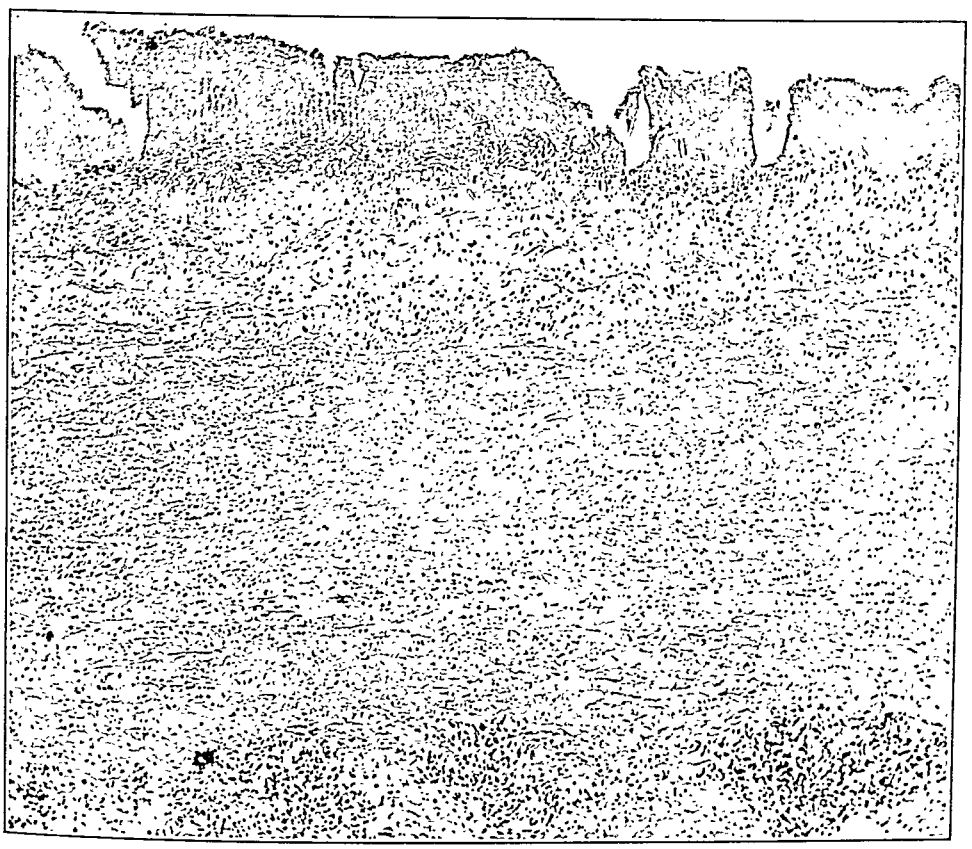
PLATE 14

FIG. 1. Case 1. Heart and aorta. Showing two of the three aortic plaques.

FIG. 2. Case 1. Aorta. H and E. Showing low power view of intima and media. Marked fibrinous infiltration of intima and perivascular cellular medial infiltration. × 40.



1

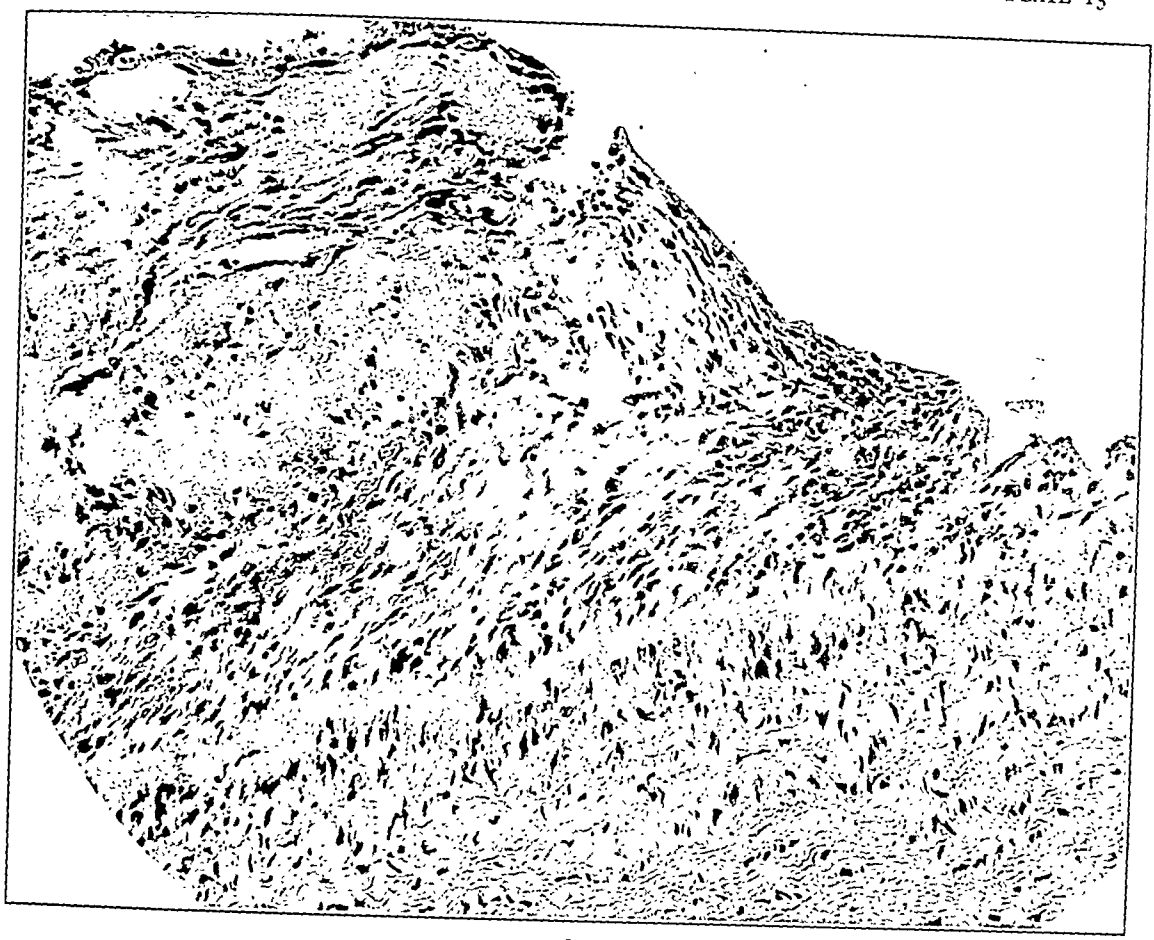


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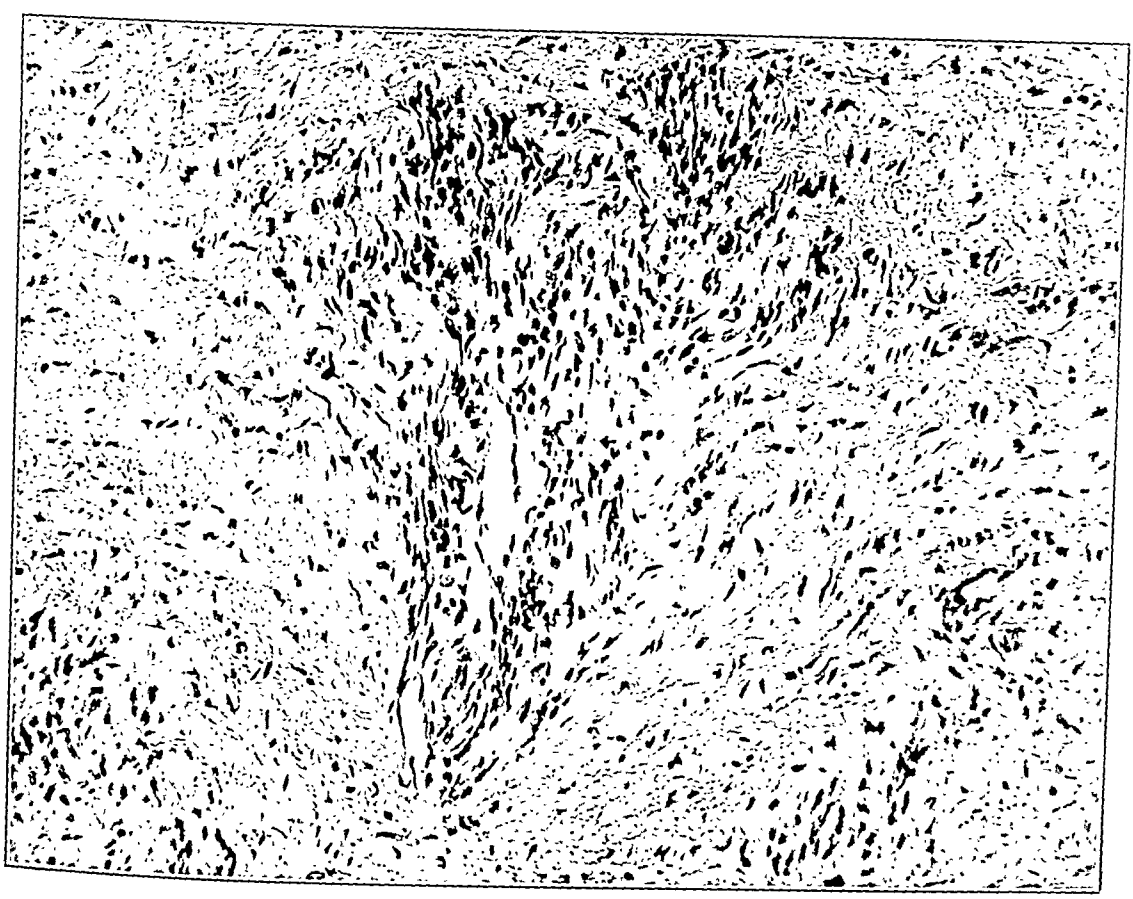
Plate 15

Fig. 3. Case 1. Aorta. H and E. Organized periphery of an intimal plaque showing characteristic endothelial-lined indentation. × 200.

Fig. 4. Case 1. Aorta. H and E. Showing the fan-like medial infiltration. × 200.



3



4

Perla and Deutch

Lesion of Aorta in Rheumatic Infections

Plate 16

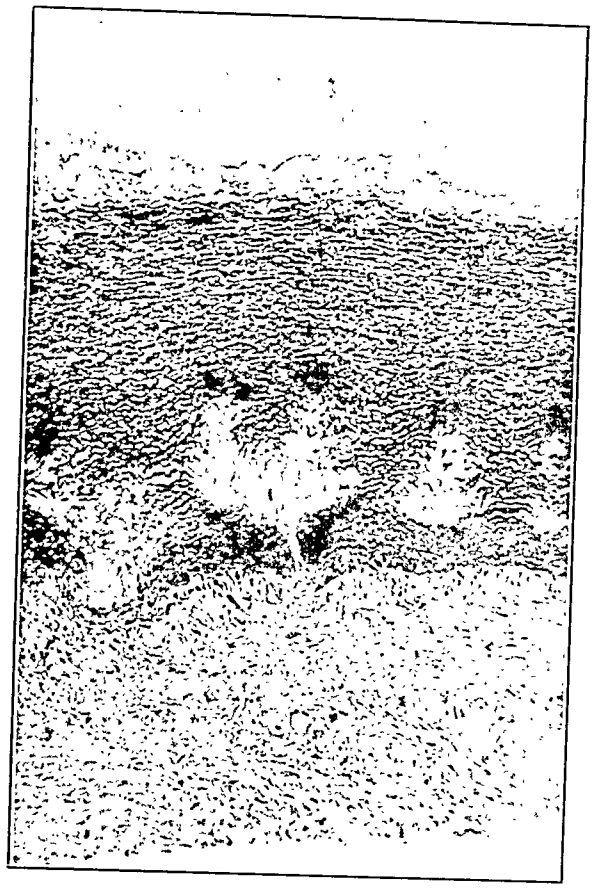
Fig. 5. Case 1. Aorta. Showing the high fibrin content of the intimal plaque and the cellular elements of intima growing into the fibrin plaque. × 40.

Fig. 6. Case 1. Aorta. Weigert's elastic tissue — Van Gieson. Extensive destructive changes in elastic tissue. × 40.

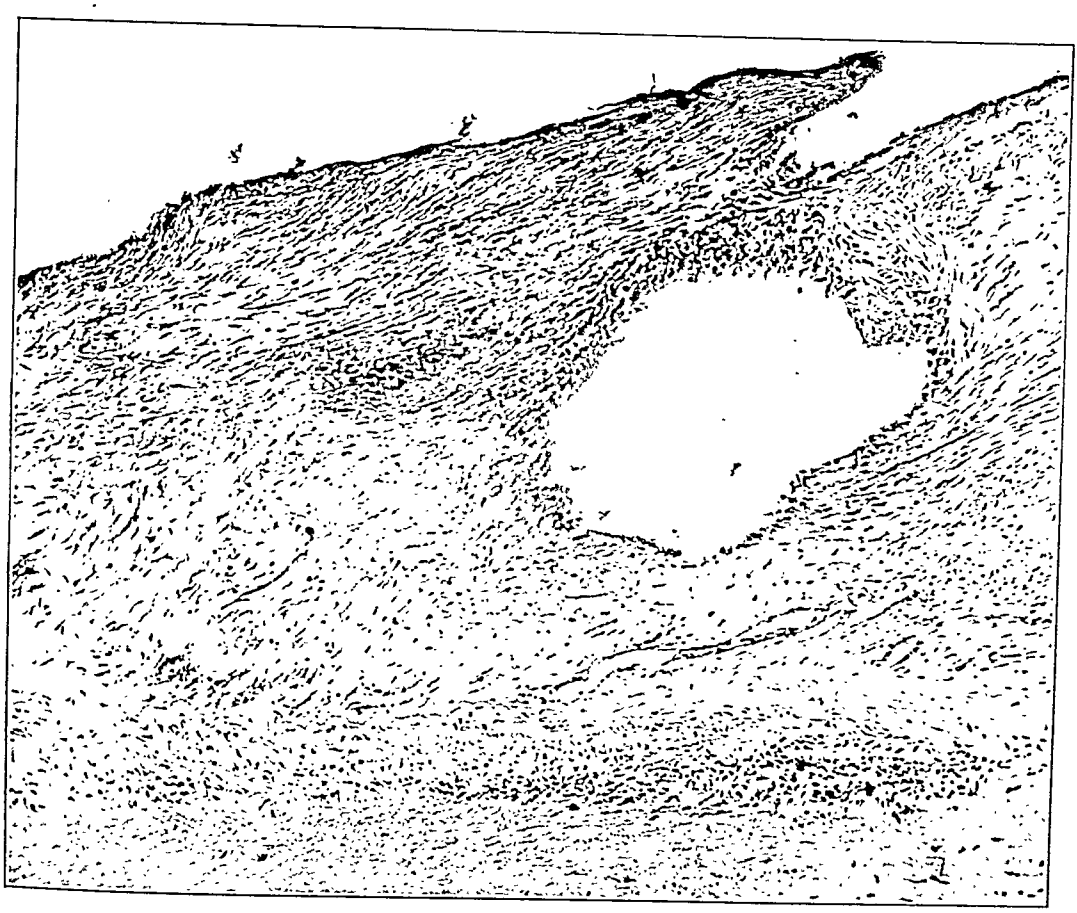
Fig. 7. Case 2. Aorta. H and E. Showing the organized intimal plaque presenting intimal indentations and cellular infiltration at base of plaque. × 40.



5



6



7

Perla and Deutch

Lesion of Aorta in Rheumatic Infections

TISSUE CHANGES ASSOCIATED WITH VITAMIN *A* DEFICIENCY IN THE RAT *

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The conception of the vitamins as specific dietary requirements rests in part upon the uniformity and reproducibility of the effects resulting from a lack of these substances in the food. Not only are food intake and growth affected but other alterations in physiology and structure can be demonstrated. It was early pointed out by Osborne and Mendel (1913) [1] that when rats are given a simple ration, adequate except for vitamin A, there ultimately develops a type of eye disease which may be cured by incorporating butter fat in the food. This was long considered the principal lesion associated with this dietary deficiency.

In a further study of the changes associated with vitamin A deficiency Mori (1922) [2] concluded that the principal lesion is an atrophy of the glandular epithelium and a loss of secretory power on the part of the lacrymal gland. He stated that the drying of the eye and the xerosis of the corneal epithelium (xerophthalmia) are entirely dependent upon the lack of glandular activity. This investigator also found cornification of the mucosa of the larynx and trachea and of the ducts of the submaxillary, sublingual and parotid glands to be present. He said that if two per cent cod liver oil be added to the diet of rats with ophthalmia the conjunctival sac becomes moistened and the xerosis disappears. At the same time the lacrymal and salivary glands become normal histologically and show evidence of secretion. In contrast to the above are the papers reported by Yudkin and Lambert (1923).[3, 4] These authors concluded that the ocular manifestations of vitamin A deficiency are dependent upon the presence of a low-grade inflammatory process which commences in the nictitating membrane and palpebral conjunctiva, and spreads to the cornea, and that the keratinization of the corneal epithelium is

* Received for publication September 1, 1928.

secondary to the inflammatory process. Infection, according to them, plays an important rôle. Wason (1921)[5] concluded that the corneal changes are due to infection. She found no pathological changes other than those in the eye.

Wolbach and Howe (1925)[6] have made an extensive study of the pathological changes throughout the body of the rat. They concluded that the primary change is in epithelium and comprises "the substitution of stratified keratinizing epithelium for normal epithelium in various parts of the respiratory tract, alimentary tract, eyes and the paraocular glands and the genito-urinary tract." They did not state definitely in what order the above systems are involved, though in their experience the epithelium of the turbinate bones and of the submaxillary gland is always affected early. They mentioned the formation of large cysts in the accessory glands at the base of the tongue and described them as being lined by keratinizing epithelium. The cysts often attain a large size, sufficient to interfere with swallowing. They did not say in what proportion of cases this lesion occurred, but they believe that the process of keratinization and cyst formation can take place in the absence of infection. The submaxillary gland has received more attention from these authors and it is their opinion that changes in this structure precede those in the eye. They concluded that the lack of secretion of the glands throughout the body does not play a primary rôle in producing the metaplasia; that infection also is a secondary manifestation; and that the lack of the single vitamin A factor is directly responsible for the metaplastic activity of the epithelium.

It has been pointed out by Goldblatt and Benischek (1927)[7] that the diets employed by Mori, and by Wolbach and Howe, were deficient in vitamins D and C as well as vitamin A. Goldblatt and Benischek, however, carried on experiments of their own and were able to reproduce the lesions described by the previous authors when vitamin A alone was lacking in the diet. They also noted the great frequency of abscesses at the base of the tongue in rats which were on a deficient diet and believed that the infectious process is "coincident with or subsequent to the epithelial metaplasia and is not responsible for the initiation of the changes." No metaplastic changes were found by them in other structures when there was none in the sublingual glands and respiratory tract. Tongue lesions have also been noted by Sherman and Munsell (1925)[8] who found

them in seventy-six per cent of rats on a diet deficient in vitamin A. The antirachitic potency was not considered in their experimental diet. According to Osborne and Mendel (1917)[9, 10] and Van Leersum (1928)[11] there is a high incidence of urolithiasis in rats deprived of vitamin A. In Van Leersum's experience accompanying infection of the kidney is uncommon.

All workers thus far are in accord in finding epithelial metaplasia to be present in vitamin A deficiency. There is still some question about the rôle of infection in the early stages of the epithelial transformation. A satisfactory correlation of the appearance of xerophthalmia with the occurrence of lesions elsewhere in the body has not been made. The order in which the several parts of the body are affected has not been established, and the sequence of events, if the deficient diet is corrected, has not been adequately described. It is therefore of interest to investigate these phases of the question with a view to correlating the sequence of events with respect to the age and weight of the animal, the relation of xerophthalmia to the appearance of other lesions in the body and the effect of correcting the deficient diet.

Experimental Procedures

Animals and Diet: White (albino) rats were used in this experiment. They were of the strain of the Connecticut Agricultural Experiment Station and were bred in the laboratory. The special diets were started as soon as the animals were at the age of 21 to 24 days. The rats were kept in individual wire cages which were supplied with false bottoms, thus preventing coprophagy. The cages and food cups were kept scrupulously clean and each cage was sterilized with steam once a week.

Individuals from each litter were divided into four groups. Group I comprised animals which were to be observed early in the course of the experiment. In Group II were those which were to be allowed to develop outspoken vitamin A deficiency or to die of the disease. The animals in Group III were to develop symptoms of vitamin A deficiency and were then to be placed on a corrected diet. Group IV comprised the positive control rats. All animals were weighed at least twice a week and sometimes every day. The food intake was determined every four days.

The diet consisted of the following:

Extracted Casein *	18 per cent
Cornstarch	51 per cent
Crisco	27 per cent
Salt Mixture (Osborne and Mendel)	4 per cent

The Crisco was melted and the other constituents thoroughly mixed with it. When allowed to cool the food was of a pasty consistency which prevented spilling. This mixture was given the rats *ad libitum*. Vitamin B was provided by giving 200 mg. daily of dried yeast which was placed in a separate dish. For vitamin D a quantity of dried yeast was irradiated under a mercury vapor lamp for one-half hour at a distance of one foot, Hess (1927).[12] Two hundred milligrams of this were fed every day. Vitamin C was lacking in the diet but it has been shown by Parsons (1920),[13] by Parsons and Hutton (1924),[14] and by Lepkovsky and Nelson (1924)[15] that the absence of this factor in the diet has no effect on the rat. The drinking water was distilled. In addition to this diet the animals in Group IV received 10 drops of cod liver oil (Squibb) each day.

Histological Technique: All tissues were fixed in 10 per cent formalin and mounted in paraffin. Hematoxylin and eosin stains were made routinely and in addition Gram and connective tissue preparations were used occasionally.

The accessory salivary glands at the base of the tongue, the submaxillary gland, the trachea and bronchi and the renal pelvis were studied in all cases. The base of the tongue has received particular attention as the lesions occur more uniformly in this region, and because of its size it is possible to make a more complete study of the tongue. Accordingly, five blocks of the tongue were mounted in each case, and from each of the blocks two to six microscopic sections were made. From the other areas one or two blocks were mounted.

Results

Clinical Course and Gross Findings: The animals on the deficient diet gained steadily for a time but always less rapidly than the control rats in the same litter. Xerophthalmia appeared on an average when the rat had been on the diet forty days, though the extremes

* 95 per cent alcohol for 2 hours; ether, 2 hours; ether, 1 hour. Dried in oven at 105° C for 48 hours.

were twenty-seven and fifty days. The sequence of events in the development of xerophthalmia were: first, a slight swelling of the eyelids accompanied by photophobia (slit-like eyes); second, a clear discharge from the eyes which sometimes became blood-tinged; third, drying of the eyes, the lids becoming glued together and the eyeball appearing to sink into its socket. Occasionally corneal ulceration and hypopyon developed. Anterior staphyloma occurred in one case.

The appearance of xerophthalmia in relation to weight loss was variable. Sometimes eye changes appeared before there was any loss of weight. Again, several grams of body weight would be lost before eye changes became manifest. Occasionally xerophthalmia appeared and the body weight continued to increase and reached a maximum level several days later. The most usual sequence was an increase in weight to a point where it remained stationary for two or three days, then, appearance of xerophthalmia and rapid decline in weight. In Group I the animals were sacrificed early in the course of the deficiency. Some were killed when a few grams of weight had been lost, others when weight had been lost and the very earliest eye changes were observed. Rat 12 was killed before any changes were observed and Rat 26 had had xerophthalmia for six days when it was sacrificed. At autopsy no gross lesions were noted in the animals in this group. In Group II all the animals had varying degrees of xerophthalmia. All showed great emaciation and every rat had abscesses at the base of the tongue from which pus could be expressed. The latter lesions probably cause pain on swallowing and may account for the terminal falling off of food intake as is suggested by Goldblatt and Benischek. The tongue and eye lesions were the only constant gross findings. The submaxillary gland was often atrophied and occasionally contained abscesses. Urinary calculi were found in some cases and where obstruction occurred pyonephrosis was present.

The animals in Group III were allowed to develop xerophthalmia. and after several days when the eye changes were outspoken and considerable weight had been lost, cod liver oil was given. Often the rats were so weak that the oil had to be given by dropper. As a rule the response to diet correction was rapid. The xerophthalmia disappeared in from one to five days and the weight curve rose abruptly. Half the animals planned for this group could not be used as compli-

cations caused the death of the rat in spite of treatment. However, Rat 7 gained weight rapidly and was apparently well when autopsy revealed an extensive unilateral pyonephrosis. Rat 18 was allowed to develop xerophthalmia twice. The second time cod liver oil did not cure, as a pyonephrosis and perinephritic abscess were present. Following cod liver oil administration the rats were sacrificed at varying intervals. In the oldest cases no gross lesions were visible except in Rat 7. In those killed soon after cod liver oil was given, tongue lesions were present.

When cod liver oil was provided from the start (Group IV) the animals gained weight rapidly, and showed no gross evidence of disease whatever even after as much as one hundred and forty-four days of the diet.

Histological Findings: The earliest changes (Table I) are found in the salivary glands at the base of the tongue. These changes consist in dilatation of the main ducts with metaplasia of the duct epithelium to the squamous keratinizing type. Infection is always present even in the earliest detectable lesion and is often found in parts of the tongue where keratinization has not appeared. The infection is characterized by a polymorphonuclear leukocytic exudate and by necrosis of the acinar epithelium.

The serous type of gland is always affected before the mucous type in the tongue, and advanced destruction of the former is always present before the latter shows definite abnormalities. The submaxillary gland is affected after the tongue has developed outspoken changes. In the submaxillary gland the same sequence of events is noted, namely, dilatation and epithelial metaplasia of the main ducts, accompanied by infection. In both the tongue and submaxillary glands it is frequently noted that only slight metaplastic changes are present while the glands are diffusely infiltrated with polymorphonuclear leukocytes. In every case the dilated ducts are filled with these cells.

Changes in the trachea, bronchi and renal pelvis follow those in the tongue and submaxillary gland. Alterations in the trachea and bronchi are inconstant. The first change is an atrophy of the surface epithelium. This is followed by a dilatation of the ducts of the glands which are filled with polymorphonuclear leukocytes. Changes in the epithelium of the renal pelvis are sometimes observed before those in the respiratory tract. The earliest alterations consist of a

TABLE I

Group I — Basal Diet

Number of rat	Initial weight	Maximum weight	Final weight	Days on diet	Eyes at death	Histological Findings				
						Tongue	Submaxillary gland	Trachea	Bronchi	Renal pelvis
	gm.	*gm.*	*gm.*							
12.....	46	150	150	47	Normal	Normal	Normal	Normal	Normal	Normal
20.....	36	112	110	45	Normal	Early metaplasia Infection	Normal	Normal	Normal	Normal
21.....	38	92	86	45	Watery discharge for one day	Early metaplasia Infection	Normal	Normal	Normal	Normal
10.....	46	132	124	51	Watery discharge for one day	Early metaplasia Infection	Few leukocytes in ducts	Atrophy of epithelium	Normal	Normal
23.....	32	102	92	49	Discharge for six days	Marked metaplasia and infection	Duct metaplasia and infection	Normal	Normal	Metaplasia Infection

piling up of the epithelium and an infiltration of polymorphonuclear leukocytes in the subepithelial tissues.

As the disease advances the ducts of the lingual glands dilate more and more, and the infection progresses so that in animals which die of vitamin A deficiency (Table II) the serous glands of the tongue are entirely destroyed by pressure and by infection, with the result that all that can be seen are large abscesses lined by squamous epithelium and filled with polymorphonuclear leukocytes, keratin and necrotic débris. In all advanced cases the epithelial lining of the abscesses is deficient at several points and the surrounding muscle is heavily infiltrated with polymorphonuclear leukocytes and shows extensive necrosis. Gram stains show the abscesses to contain large masses of bacteria in which Gram-positive cocci predominate. In these late cases the submaxillary gland is nearly always involved though occasionally no change other than atrophy is noted. The most frequent finding is large epithelium-lined spaces filled with polymorphonuclear leukocytes while the rest of the gland is diffusely infiltrated with the cells. It is a picture similar to that in the tongue. In a few instances the entire submaxillary gland is converted into an abscess.

The late manifestations in the trachea and bronchi are not uniform as the changes vary all the way from simple atrophy to metaplasia of the surface epithelium and cyst formation in the glands with extensive infection of the surrounding tissue. The epithelium of the renal pelvis is always involved in the late cases. Here the epithelial layer is deeper, keratinization is marked and in some instances the entire renal pelvis is filled with keratin and leukocytes. In several cases calculi blocked the urinary outflow in either the bladder or ureter with the development of pyoureter and pyonephrosis. Two instances of perinephritic abscess developed following rupture of obstructed ureters. The tendency toward hyperplastic activity of the epithelium which has undergone transformation to the squamous type is noted by Wolbach and Howe (1925).[6] This is sometimes very striking and occurs with particular frequency in the tongue and renal pelvis. In the tongue the circle of epithelium surrounding one of the abscesses may be very broad and the outer layers often contain mitotic figures. Occasionally long, finger-like epithelial processes extend into the surrounding tissue and with distortion due to infection the picture may be one difficult to distinguish from malignancy.

TABLE II

Group II — Basal Diet

Number of rat	Initial weight	Maximum weight	Final weight	Days on diet	Eyes at death	Histological Findings				
						Tongue	Submaxillary gland	Trachea	Bronchi	Renal pelvis
	gm.	*gm.*	*gm.*							
14.....	35	102	74	42	Xerophthalmia	Metaplasia Abscess	Normal	Atrophy of epithelium	Atrophy of epithelium	Metaplasia Pyonephritis
2.....	36	107	94	48	Xerophthalmia Hypopyon	Metaplasia Abscess	Slight metaplasia. Slight infection	Metaplasia Infection	Metaplasia Infection	Slight metaplasia Infection
8.....	44	88	72	56	Xerophthalmia	Metaplasia Abscess	Atrophy	Normal	Normal	Early metaplasia Infection
3.....	38	96	82	57	Xerophthalmia Hypopyon	Metaplasia Abscess	Slight metaplasia Infection	Atrophy of epithelium	Atrophy of epithelium	Metaplasia Infection
6.....	45	110	82	61	Xerophthalmia	Metaplasia Abscess	Early metaplasia Infection	Atrophy of epithelium	Normal	Metaplasia Infection
27.....	32	92	68	63	Xerophthalmia	Metaplasia Abscess	Metaplasia Abscess	Atrophy of epithelium	Normal	Metaplasia Pyonephrosis
4.....	45	126	80	75	Xerophthalmia Corneal ulceration	Metaplasia Abscess	Metaplasia Abscess	Atrophy of epithelium	Atrophy of epithelium	Metaplasia Infection

TABLE III

Group III — Basal Diet + Cod Liver Oil after Ophthalmia and Loss in Weight

Number of rat	Initial weight	Maximum weight before oil	Weight when oil given	Final weight	Days on oil	Eyes at death	Histological Findings				
							Tongue	Submaxillary gland	Trachea	Bronchi	Renal pelvis
	gm.	*gm.*	*gm.*	*gm.*							
17....	33	120	106	112	4	Normal	Metaplasia Abscess Fibrosis regeneration	Metaplasia of ducts. Infection	Normal	Normal	Hyperplasia Infection
25....	30	56	56	90	14	Normal	Metaplasia Small abscess Fibrosis	Normal	Normal	Normal	Hyperplasia Infection
18....	36	116	98	152	5	Xeroph-thalmia	Metaplasia Abscess	Abscess	Normal	Normal	Metaplasia Pyonephrosis Perinephritic abscess
13....	49	112	112	164	56	Normal	Normal	Normal	Slight infection	Peribronchial fibrosis	Infection
7....	44	124	88	180	91	Normal	Slight meta-plasia Chronic infection	Normal	Normal	Peribronchial fibrosis	Pyonephrosis Perinephritic abscess

In the renal pelvis the hyperplastic changes always overshadow the keratinization. The lining epithelium becomes piled up into a broad layer and columns or sheets of epithelial cells extend into the subepithelial tissue.

After the administration of cod liver oil to rats which have developed xerophthalmia (Table III) the healing process depends upon the extent of destruction of the particular portion by the infectious

TABLE IV

Group IV — Basal Diet + Cod Liver Oil from Beginning

Number of rat	Initial weight	Maximum weight	Final weight	Days on diet	Lesions
	gm.	*gm.*	*gm.*	*gm.*	
24.....	30	144	144	47	None
22.....	30	110	110	49	None
19.....	33	152	152	49	None
9.....	47	166	166	51	None
26.....	30	140	140	65	None
1.....	47	212	212	73	Bronchitis; bronchopneumonia
15.....	38	152	126	100	None
5.....	46	170	170	106	None
11.....	40	214	200	144	None

process. In the tongue there may be almost complete regeneration of the glandular epithelium with only a few scarred areas remaining. The submaxillary gland often shows no evidence of previous damage. The trachea and bronchi may appear perfectly normal though there is usually some fibrosis of the surrounding tissue. The epithelium of the renal pelvis has shown evidence of hyperplasia with keratinization and infection as long as ninety-one days after the animal has gained weight and is apparently well. The usual picture for as much as two weeks after correction of the diet is the persistence of infection and abnormal epithelium in the tongue and kidney and apparently normal epithelium in the trachea and bronchi. At this

stage there is usually extensive fibroblastic activity and round cell infiltration surrounding the tongue abscesses with evidence of epithelial regeneration in less severely affected portions of the gland. In one animal the only evidence of previous damage to the tongue is a collection of small round cells and endothelial leukocytes replacing part of one of the lobules of the lingual gland.

The control animals (Table IV) were all well nourished at the time of autopsy. The sections from one rat show slight bronchitis and bronchopneumonia. No other abnormal findings were observed in any of the rats in this group.

The tissue changes in the various structures studied have been correlated with age and weight changes and are summarized in the four tables.

Summary

The principal changes associated with vitamin A deficiency in rats are a metaplasia of cuboidal or columnar epithelium in certain parts of the body, epithelial hyperplasia in various structures and infection.

The metaplastic changes involve the following structures in order: the sublingual glands, the submaxillary glands, the epithelium of the renal pelvis and of the trachea and bronchi. The tongue is regularly involved before xerophthalmia appears. The serous type of sublingual gland is the first to be affected. The lesion in the tongue and submaxillary gland begins with a dilatation of the ducts and a metaplasia of the lining epithelium accompanied by infection. In late cases the glandular tissue may be entirely destroyed by pressure from the dilated ducts and by necrosis due to infection. The submaxillary gland is not involved as constantly as the tongue. The epithelium of the renal pelvis may be involved quite early. Metaplasia and infection are always present in the advanced cases. Renal calculi are prone to occur and when obstruction to the urinary outflow is present pyonephrosis develops which is sometimes followed by perinephritic abscess. Epithelial metaplasia of the trachea and bronchi is not common. The most usual finding is an atrophy of the lining cells.

Epithelial hyperplasia is striking in the tongue and renal pelvis. In the latter the hyperplasia overshadows the keratinizing process.

Infection is always present even in the earliest stages and in late cases dominates the picture. No metaplastic activity has been seen without an accompanying infection, but infection has been observed in parts where metaplasia is absent.

If the results of the dietary deficiency are not too severe, xerophthalmia clears rapidly with the administration of cod liver oil and the weight curve rises abruptly. The extent of healing in the various organs depends largely upon the amount of destruction due to infection which is present. Following cod liver oil administration abnormal epithelium and chronic or acute infection persist in the tongue and renal pelvis when the rat is apparently healthy.

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DESCRIPTION OF PLATES

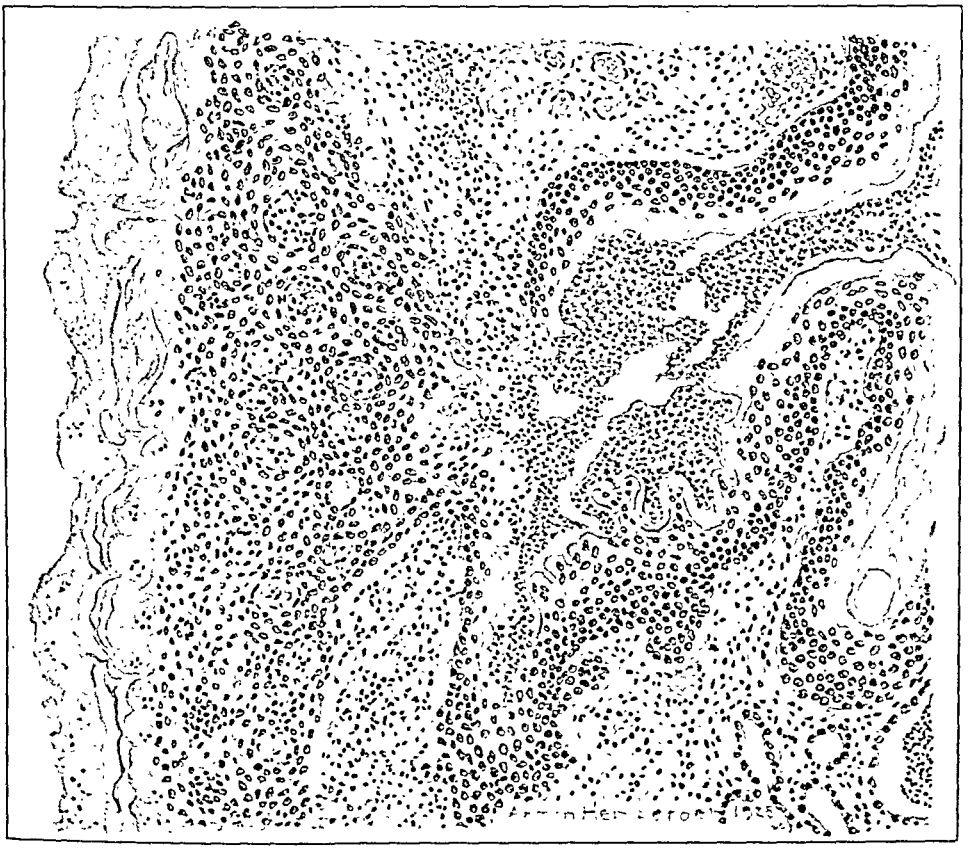
PLATE 17

FIG 1. Rat 21. Serous portion of sublingual gland. Early lesion. Dilatation of ducts with metaplasia of the lining epithelium. Polymorphonuclear leukocytic exudate in ducts and adjacent glandular tissue. ×125.

FIG. 2. Rat 6. Cross-section of tongue. Advanced lesion. Great dilatation of ducts with epithelial metaplasia. Exudate in ducts and surrounding muscle. Necrosis of muscle and supporting tissue. Hyperplasia of the surface epithelium. Drawing ×1150.



1



2

Tyson and Smith Vitamin A Deficiency in Rat

Plate 18

FIG. 3. Rat 25. Hyperplasia of epithelium of renal pelvis. Exudate in epithelial layer and subepithelial tissues. ×125.

FIG. 4. Rat 25. Abscess of tongue partly lined by metaplastic epithelium. Keratosis not marked. Extensive fibroblastic activity surrounding the abscess. ×65.



3



4

STUDIES ON COMPENSATORY HYPERTROPHY OF THE THYROID GLAND*

VIII. A COMPARISON BETWEEN THE EFFECT OF ADMINISTRATION OF THYROXIN, THYROID AND ANTERIOR PITUITARY SUBSTANCE ON THE COMPENSATORY HYPERTROPHY OF THE THYROID GLAND IN THE GUINEA PIG

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In a previous investigation [1] we have shown that feeding of guinea pigs with thyroid tablets (Armour & Co.) prevents the compensatory hypertrophy of the thyroid gland. Subsequently in association with E. E. Kaplan [2] we found that feeding with anterior pituitary substance (Armour & Co.) acts in a similar manner to the thyroid substance; it also prevents compensatory hypertrophy. In the present investigation we wished (1) to confirm our previous results concerning the action of anterior pituitary substance, as this seemed desirable in view of the prevalent belief that feeding with this substance exerts no definite action; (2) to compare with the effect of feeding thyroid tablets that following the administration of thyroxin, it being at present a debated question whether or not thyroid substance contains active substances in addition to thyroxin; (3) to determine the relative potency of these various substances as far as their action on the compensatory hypertophy of the thyroid is concerned.

I. THE EFFECT OF FEEDING WITH ANTERIOR PITUITARY SUBSTANCE

In eleven guinea pigs one whole lobe and three-fourths of the second lobe of the thyroid gland were removed. Two days after the operation each animal received by mouth five grains of anterior pituitary substance in tablet form (Armour & Co.) and this dose was continued daily. Thirty to thirty-two days after operation the remaining parts of the gland were removed. The experiments were carried out during January and February. The initial weight of the

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guinea pigs varied between 342 and 446 gm. In seven animals there was a considerable increase in weight during this period; in one animal the weight remained approximately stationary and in three others the weight at the end of the experiment was not recorded. In order to render unnecessary detailed descriptions of our findings we shall make use of a system of grades which we introduced previously[3] for this purpose. In eight guinea pigs the grade indicating the hypertrophy or lack of hypertrophy varied between 5 and 5.25, while in three additional guinea pigs the grade varied between 4.85 and 5. There was therefore no hypertrophy found in these glands, notwithstanding a decided increase in weight during the period of the experiment, in the majority of animals. However, feeding with anterior pituitary substance not only prevented hypertrophy from taking place, but in this group the acini were markedly below the average size, contained rather solid and slightly retracted colloid, epithelium somewhat below medium size and rather infrequent phagocytes which were usually small and unable to dissolve the hard colloid. While these characteristics prevailed on the whole, there were found in some glands areas where the acini were somewhat larger and the acinus cells likewise higher, without, however, becoming definitely hypertrophic. In such acini, also, the colloid was at times somewhat less hard, adhering to the epithelium by means of threads between which vacuoles were included. Occasionally a larger number of phagocytes were found in the colloid of such acini. In some places an intermediate condition existed.

In control animals in which also the greater part of the thyroid gland had been removed, but in which the pituitary extract was not administered, the size of the epithelial cells was higher on the average, the colloid softer and the peripheral vacuolar zones or solution processes in the colloid were seen more frequently. The average size of the acini was likewise greater and the acini showed often an irregular outline in the controls, in contrast with the more regular outline of the usually smaller acini in the glands of the guinea pigs fed with pituitary substance. Whereas in the glands of the latter animals, mitoses, if they occurred at all, were very rare, they were observed much more frequently in the controls and the phagocytes were here on the average larger and more numerous. The breaking through of the walls of neighboring acini and the development of papillae were encountered more often in the controls although they

occurred also in the pituitary-fed guinea pigs. In addition there were some cases among the controls in which the hypertrophy was quite pronounced, whereas this did not occur in the pituitary group. In comparing thus the condition of the thyroid remnants of animals fed with pituitary substance and of controls, we must take into consideration the fact that in this series a majority of the control animals had lost weight, or at least had not gained in weight during the course of the experiment, while a majority of the guinea pigs in the pituitary group had gained in weight, and thus the real difference between the pituitary-fed and the control animals was greater than is indicated in these experiments.

II. The Effect of Feeding Thyroid on Compensatory Hypertrophy of the Thyroid Gland

Eight guinea pigs with an initial weight varying between 315 and 352 gm. were used in this series. Beginning two days after total extirpation of one lobe and three-fourths of the other lobe of the thyroid gland, some of the animals were fed with two to four tablets (Armour & Co.) of 1/10 grain of thyroid substance; in others four tablets were administered during the first week or two, then the dose was reduced to two tablets. In one case six tablets were given during the first thirteen days, when the dose was reduced to three tablets. In general our aim was to administer thyroid substance in such a way that the guinea pigs gained in weight during the course of the experiment. In several guinea pigs therefore the administration was interrupted during the experiment for a few days in order to prevent a loss of weight. As a result of these precautions we succeeded in all the animals except one, which lost 25 gm., in obtaining a gain in weight during the course of the experiment which extended over a period of thirty to thirty-five days.

Notwithstanding this gain in weight the remnants of the thyroid gland showed inactive gland tissue. The acini were mostly of small size and were lined with very flat, or low to medium epithelium. However, in some areas the acini were a little larger. The colloid was usually solid and slightly retracted. Rarely there was a somewhat softer colloid adherent to the epithelium and a peripheral vacuolar zone was observed, or a greater retraction of the colloid had taken place indicating a more marked solution process. The

number of phagocytes contained in the colloid was on the whole very small, especially in acini lined with flat epithelium; where the epithelium was of medium size the phagocytes were often slightly more numerous. Usually they did not succeed in digesting the hard colloid but occasionally they seemed to cut out some balls of it. The grade characterizing such a condition of the thyroid gland lies somewhere between 5 and 6, indicating a tendency to shrink on the part of the acini and of the epithelium and an increasing hardness of the colloid.

In general this reduction in the size of the glandular elements and the corresponding increase in the hardness of the colloid was greater in the series fed with thyroid tablets than in those fed with anterior pituitary substance, notwithstanding the fact that the gain in weight was equally present in both series. It is, therefore, very probable that the administration of thyroid substance is more effective than the anterior pituitary in preventing hypertrophy of the thyroid gland and in reducing its size and function, the former producing the structural signs of functional inactivity of the gland to a still higher degree than the former.

III. The Effect of Administration of Thyroxin

A group of eight guinea pigs were treated in the same way as the two former groups, except that instead of being fed with thyroid tablets they received by mouth tablets containing 1/320 of a grain each of thyroxin. In some cases four to six tablets were administered in the beginning but after 9 to 12 days this dose was reduced to one-half this amount. In one animal as little as one tablet was given daily. In several guinea pigs the administration of thyroxin had to be interrupted for several days because this substance caused a loss of weight, the weight in the beginning of the experiment varying between 321 and 409 gm.; only three of the animals gained in weight during the course of the experiment, while four remained about stationary, and one decreased considerably. The last-mentioned guinea pig died twenty-four days after the removal of the greater part of the thyroid gland, and inasmuch as no remnants of the thyroid gland were found at the time of examination it had to be excluded in considering the results. There thus remained seven guinea pigs in none of which was any hypertrophy noted. In gen-

eral the condition of the remaining parts of the glands was similar to that seen after feeding of thyroid tablets; the average grade was also about the same in both cases ranging somewhere between 5 and 6. The small-sized acini predominated, but there were seen some acini of medium size. The height of the epithelial cells was usually low or it ranged between low and medium. The colloid was generally hard and slightly retracted but occasionally it was somewhat more retracted as a result of a certain degree of softening, or it adhered with threads to the epithelium and showed a vacuolar zone in the periphery. The number of the phagocytes was on the whole very small; only rarely some larger-sized phagocytes were seen in the colloid or occasionally even an acinus was filled with small phagocytes. Collections of lymphocytes were not seen in the stroma.

We find then that thyroxin acts in the same way as thyroid substance when fed to guinea pigs, both preventing hypertrophy and reducing the activity of the thyroid gland to about equal degrees.

Discussion and Conclusions

These investigations confirm our previous conclusions concerning the effect of feeding anterior pituitary gland substance (Armour & Co.) on the thyroid in guinea pigs; it prevents compensatory hypertrophy of this organ and tends to produce in it a condition corresponding to a resting stage. In general it decreases the size of the acini and the acinus epithelium becomes lower; it also causes a hardening of the colloid. In this respect, therefore, administration of anterior pituitary substance resembles in its action feeding with thyroid gland substance; however, it is not quite so effective as the latter. Feeding with anterior pituitary substance does not reduce the average size of the acini and of epithelial cells to the same extent as does thyroid substance. In the former instance there may still be found in various glands or in certain places in a single gland, a greater number of medium-sized acini and epithelial cells; the colloid may at such points be a little softer, a greater size of acini and of epithelial cells being usually associated with a decreased hardness of colloid. In accordance with the reduction in activity indicated by the structural changes resulting from administration of these two substances there is a diminution in the number of phagocytes found, and the phagocytes that do enter the colloid are usually small in size and do not succeed in causing a localized solution of the colloid;

furthermore, the collections of lymphocytes which are occasionally observed in controls and especially in the iodine-fed guinea pigs are absent in these thyroid glands, a further evidence of reduced gland activity. The solution of walls separating neighboring acini, although it does occur, is rare.

We see then that feeding with anterior pituitary substance as well as with thyroid substance tends not only to prevent compensatory hypertrophy in the guinea pig, but also to reduce the activity of the gland below the level of that of a normal gland; in this respect, however, thyroid substance is in all probability somewhat more effective. In addition, our experiments show that thyroxin very much resembles thyroid substance in its action, at least in the prevention of compensatory hypertrophy, as well as in the production of those structural changes which indicate functional inactivity. We may therefore conclude that it is the thyroid hormone itself and not another substance mixed with it which exerts the typical effect on the thyroid gland. As to the ingredient in Armour's preparation of anterior pituitary substance which exerts this effect similar to that of thyroxin, we are not in a position to make a definite statement, except that we know that it cannot be due to the admixture of inorganic iodine because the latter does not depress the activity of the thyroid gland, but on the contrary stimulates it; but whether an organic iodine substance resembling thyroxin in its action is responsible for this effect is a question that must be left open at the present time.

We find thus certain structural features which are correlated and which form a complex indicative of thyroid inactivity. The main features of this condition are: (1) small size and regularity in shape of acini; (2) relative flatness of epithelium; (3) decrease in number of mitoses; (4) hardness of colloid; (5) diminution in number, size and activity of the phagocytes; (6) probable decrease in number of lymphocytes found in these glands. It is of interest to contrast with this combination of features the condition produced by factors causing a stimulation of the gland. Here we find, at least in certain areas: (1) larger than medium-sized and often irregular acini; (2) higher epithelium; (3) increase in number of mitoses; (4) softness of colloid; (5) increase in number, size and activity of phagocytes; (6) a probable increase in accumulation of lymphocytes and in number of spurs and papillae reaching into the cavity of the acini. Compen-

satory hypertrophy and other types of hypertrophy lead to this latter condition; at least certain of these features are produced also by the administration of potassium iodide until such a time as secondary changes take place.

The similarity in the action of thyroid hormone and of anterior pituitary substance which we thus find, is in harmony with some other facts which likewise point to a relationship between these two substances. It is known that removal of the thyroid gland sets in motion certain hypertrophic processes in the anterior lobe of the pituitary gland. Thyroid substance and thyroxin prevent compensatory hypertrophy and change the structure of an active into that of an inactive gland by supplying that constituent, the lack of which sets into motion hypertrophic changes; and we must assume that the anterior pituitary also supplies a certain constituent which in some respects acts similarly to that given off by the thyroid gland, preventing compensatory hypertrophy and inducing structural changes which lead to cessation of gland functions. Thus both functional activity and structural features are coördinated in the effect of thyroid, anterior pituitary substance and thyroxin administration; after KI such a coördination also exists but in this case it produces results of an opposite character.

It is interesting to refer briefly in this connection to the comparative effects of thyroid and anterior pituitary substance on the metamorphosis of amphibian larvae. It is well known that feeding thyroid substance accelerates this process. Now, Hogben [4] found that injection of anterior pituitary substance (Armour & Co.) also induces metamorphosis in the Mexican axolotol. Smith [5] working with the Colorado axolotol likewise obtained positive results in feeding this preparation, but believed this effect to have been caused by an admixture of thyroid gland to Armour's anterior pituitary substance. However, Spaul [6] as well as Uhlenhuth and Schwartzbach [7] attributed the metamorphosis induced by injection of anterior pituitary substance to the pituitary preparation as such; in particular Uhlenhuth and Schwartzbach could show that the metamorphosis in this case depends in all probability upon a stimulating effect which the injected material exerts on the thyroid gland. It is this marked stimulation of the thyroid gland in the axolotol which distinguishes sharply the effect of anterior pituitary in the axolotol and in the guinea pig; in the latter animal we have shown that this substance

causes a depression in the thyroid gland; therefore its action on the guinea pig is the reverse of the action in amphibia as observed by Uhlenhuth and Schwartzbach. The results obtained by the last-named authors concerning the stimulative effect of anterior pituitary substance on the thyroid gland are in agreement with some observations of Allen.[8] We must therefore consider the possibility that the action of anterior pituitary substance on the thyroid gland in mammals and in amphibia may be different in kind, or that in both cases different constituents of the anterior pituitary glands are active.

Summary

1. The inhibiting effect of feeding anterior pituitary substance on compensatory hypertrophy of the thyroid gland previously found by Loeb and Kaplan is confirmed.

2. Anterior pituitary substance, thyroid substance and thyroxin all produce similar effects, not only preventing compensatory hypertrophy of the thyroid gland but also tending to produce changes in the gland that signify a resting condition.

3. There probably exist quantitative differences between the effectiveness of these substances, anterior pituitary extract being somewhat less effective than the others.

4. This similarity in the effects of thyroid and anterior pituitary substances on the thyroid gland is in accordance with certain other similarities which have been found to exist between these substances, but is not in harmony with the action of anterior pituitary observed in larvae of Urodele amphibians. It is suggested that different substances may perhaps be responsible for the effects exerted by administration of anterior pituitary substance under different conditions.

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STUDIES ON COMPENSATORY HYPERTROPHY OF THE THYROID GLAND *

IX. THE INFLUENCE OF VARIATIONS IN SIZE OF THE REMAINING PART OF THE GLAND, IN MODE OF ADMINISTRATION AND IN QUANTITY OF POTASSIUM IODIDE ON THE HYPERTROPHY OF THE THYROID IN THE GUINEA PIG

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In former communications we have shown that in guinea pigs in which the greater part of the thyroid gland had been removed, so that only about one-third to one-fifth of one lobe was left, the feeding of potassium iodide during a period of four to five weeks following the operation not only did not prevent the establishment of compensatory hypertrophy in the remaining gland tissue but, on the contrary, tended to increase the number of cases in which hypertrophy occurred and furthermore to intensify in general the degree of the hypertrophy.[1, 2] The view has been expressed by Marine [3] that if as much as one-half of one lobe is left, instead of a smaller part, administration of potassium iodide prevents hypertrophy. In our new series, therefore, as much as one-half of one lobe of thyroid remained in the animal. In addition we thought it of interest to determine the effect of variations in the amount of KI given following the operation. For this purpose we administered the same quantities of this substance which subsequently Rabinovitch used in order to test the effect of variations in the dose of KI given on the intensity of mitotic cell proliferation in the thyroid of the guinea pig. Rabinovitch found a definite relationship between the amount of iodine administered to the animals daily and the resulting cell multiplication. Furthermore, we wished to determine whether intraperitoneal injection of KI has the same effect as feeding of this substance by mouth.

We carried out two series of experiments; in both the examination took place usually thirty to thirty-two days following the operation, except in a few experiments of the second series in which it occurred

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as early as twenty days following extirpation of the gland. In the first series, carried out in the spring of 1926, the iodide was fed by mouth, whereas in the second series, carried out in the early part of 1928, intraperitoneal injections were given daily to the guinea pigs. In both series some of the animals received 0.01, others 0.05 and a third group as much as 0.1 gm. KI daily, beginning one or two days following the operation. In addition there was in each series a control group of guinea pigs which were treated in the same way as the other animals except that KI was not administered following the extirpation of the gland. In describing the results obtained we shall make use of the system of grades introduced by us in a former paper.[2] In doing so we must, however, take cognizance of the fact that the iodide induces certain changes in the gland tissue of the hypertrophying gland which are not different in principle, but which differ from those taking place in the controls as far as their intensity is concerned. Prominent among these changes is a tendency to a more rapid and complete softening and liquefaction of the colloid in the iodized guinea pigs. Furthermore, there is in many acini a delay in absorption of this liquefied material and thus subsequently a distension of the acini associated with secondary pressure effects may take place in the gland. In estimating the relative degree of compensatory hypertrophy in the iodized and control guinea pigs, we must take this factor into consideration.

Series I. KI Fed to Guinea Pigs in Form of Pills

Control Animals: Ten guinea pigs were used as controls; their initial weight varied between 413 and 540 gm., with one exception in which the weight was 325 gm. The grades of hypertrophy were: 4.50; 3.80; 4.90; 4.25; 4.90; 5.50; 3.85; 4.40; 5.0. One of the animals died prematurely; it had the grade 5.0. The average degree of hypertrophy was therefore relatively low, 4.70. We have thus far found two variable factors which influence the degree of hypertrophy in guinea pigs in which an equal amount of thyroid gland has been removed: namely, (1) the season of the year in which the experiment is carried out — during the summer months the hypertrophy is on the average less than during the rest of the year; (2) a gain in weight during the course of the experiment; animals which gain have a higher average of hypertrophy than those which do not gain weight. There are probably still other factors involved which need further

analysis. In this case the failure of the animals to gain weight during the experiment seems largely to account for the low degree of hypertrophy obtained. Of the ten animals, only three were heavier at the end of the experiment than in the beginning and two of these showed the highest grades; one, which gained only a few grams, is included in the stationary class, comprising three guinea pigs. Five animals showed a decided loss of weight during the experiment; they showed very little or no hypertrophy. As we shall see later the hypertrophy was much more pronounced in the control animals of the second series, which showed a much greater gain in weight during the experiment than the animals of the first series.

Guinea Pigs Fed with 0.01 gm. KI Daily: Eight guinea pigs were used in this group. The average grade of hypertrophy was 4.0, and the individual grades were 4.0; 3.50; 4.50; 4.25; 4.50; 3.50; 4.50; 3.25. The degree of hypertrophy is therefore decidedly higher here than in the controls. We cannot attribute the differences in hypertrophy found in this series entirely to the KI action, inasmuch as in this group six guinea pigs showed a moderate gain in weight, amounting on the average to about 20 to 40 gm. while of the two remaining animals one lost weight and the second remained about stationary. However, the structural features of KI feeding were clearly present in these animals and we may attribute, in part at least, their increase in hypertrophy over that of the controls to the administration of KI.

Guinea Pigs Fed with 0.05 gm. KI Daily: Nine guinea pigs, the initial weight of which ranged between 470 and 538 gm., were used in this group. All of these animals, except two, gained in weight during the course of the experiment. In the two which lost weight the hypertrophy was very slight, the grades being 4.90 and 4.50 respectively; in the others the grades were as follows: 4.0; 3.0; 3.0; 3.25; 4.0; 3.25; 4.75. The average grade in this group was 3.85. Again the hypertrophy was distinctly higher than in the controls and even than in the preceding group of iodized animals.

Guinea Pigs Fed with 0.1 gm. KI Daily: Eight guinea pigs, with an initial weight varying between 450 and 560 gm. served for these experiments. Three of these animals lost weight; the remaining five showed a stationary weight or a very slight gain. The average grade in this group was 4.55; the grades of the animals which lost weight were on the average lower than those of the others.

There is reason for assuming that the low degree of hypertrophy in this group is at least partly due to the unfavorable weight balance in these guinea pigs; but it is also possible that such a large dose of KI, corresponding approximately to a daily dose of 12 to 15 gm. of this substance in an adult man, has either, in some unknown way, an injurious effect upon the organism, or that it causes an excess thyroxin formation leading to a loss in weight and indirectly diminishing the activity of the thyroid gland, thus causing the diminution in hypertrophy observed.

However, when in this group hypertrophy is present the characteristic features of the latter do not differ from those usually observed, but on the average less hypertrophy occurred. The acini were smaller and the colloid more solid; yet the phagocytes were increased in number even in places in which only a trace of hypertrophy could be found, although they were not increased where hypertrophy was completely lacking. As a result of the increased solution processes in the colloid, pressure effects and occasional perforations of the walls separating adjoining acini, due to increased pressure, were observed. Here again the solution of the colloid took place by means of an agent, presumably of an enzymatic nature, acting diffusely as well as by means of phagocytes acting locally.

Series II. KI Injected Intraperitoneally

In this series the same quantities of KI, which in the first series were fed to guinea pigs, were injected intraperitoneally.

Control Animals: The initial weight in this group varied between 305 and 405 gm. except in two guinea pigs in which it was somewhat lower. Two animals examined after twenty days showed definite hypertrophy with the grades 3.0 and 3.20 and with structural changes characteristic of compensatory hypertrophy; both of these guinea pigs gained 40 to 50 gm. in weight during the course of the experiment.

Four guinea pigs were examined after thirty days, but one of these must be excluded from the list because it showed, evidently as the result of a local infection, infiltration of the gland tissue and the surrounding capsule with many polymorphonuclear leucocytes and with lymphocytes. The colloid in this case was largely liquefied and therefore increased in volume; thus pressure was exerted on the walls of the acini, but notwithstanding these effects there were still

many mitoses as well as other signs of hypertrophy observed. The grades of the two other guinea pigs, that had gained considerably in weight, were 2.80 and 3.75, whereas in an animal with almost stationary weight the hypertrophy was almost lacking, the grade being 4.75.

We may refer here to three additional control experiments carried out recently in which only one-third of one lobe had been left behind, and in which the examination took place thirty days following operation. The initial weight of these animals was between 400 and 500 gm. and they gained considerably in weight during the course of the experiment. They showed moderate degrees of hypertrophy, their grades varying between 3.50 and 3.90. While, as usual, the changes in these gland remnants were in principle similar to those observed in the iodized guinea pigs, still the typical quantitative differences, to which we have referred, were again noticeable; in particular, the marked variegation, characteristic of the KI animals, was here lacking and the colloid showed, on the whole, much less pronounced solution processes, although in individual acini the same conditions could be found as in the iodized guinea pigs.

Guinea Pigs Injected Daily with 0.01 gm. KI: The initial weight of the four animals in this group, two of which were examined after twenty days and two after thirty days, varied between 333 and 438 gm. Three of these guinea pigs gained in weight and only one, examined after thirty days, remained stationary. All the animals showed distinctly hypertrophic thyroid remnants; after twenty days the grades were 3.40 and 3.50 and after thirty days the grade was 3.0 in both animals. Lack of gain in weight thus did not prevent hypertrophy. The effects of iodine were typical in the thyroid remnants of these animals: they consisted of softening and solution of colloid, hypertrophy of epithelium with secondary flattening as the result of pressure, perforation of walls (except in a case where the connective tissue between the acini was increased and thus prevented perforation) and in general there was found the characteristic variegated appearance of the thyroid gland. It is of interest to note that these conditions were present after twenty, as well as after thirty days.

Guinea Pigs Injected Daily with 0.05 gm. KI: Of the four animals used in this group, two were examined after twenty, and two after thirty days; the initial weight of these guinea pigs varied between

308 and 420 gm. and all of them gained in weight during the course of the experiment. The gland remnants showed in each case distinct hypertrophy, the grades after twenty days being approximately 2.75 and after thirty days, 2.0 and 2.75.

The histological picture was characteristic of KI administration, after twenty, as well as after thirty days following operation. One case in particular, examined after thirty days, showed a very pronounced hypertrophy in that the whole center of the gland remnant had been converted into a system of irregular slits lined with very hypertrophic epithelium; the greater part of the colloid had been absorbed in these acini.

Guinea Pigs Injected Daily with 0.1 gm. KI: Of the four animals in this group, in two the examination took place after twenty days and in the other two after thirty and thirty-one days, respectively, following operation. The initial weight in these guinea pigs varied between 320 and 435 gm. In the animal examined after twenty days, which had lost in weight, the grade was 4.90, indicating that there was at best only a trace of hypertrophy. In the second guinea pig, which had remained stationary in weight, the grade was 3.90. The epithelium was hypertrophic; the colloid slightly softened and adherent to the epithelium with long threads. There were some groups of acini with pale colloid and hypertrophic epithelium, and on the other hand, some peripheral non-hypertrophic acini with hard colloid. In some acini the colloid had been eaten up by phagocytes. In the animal examined after thirty-one days, the weight had remained stationary and the grade was 3.50, while in the third guinea pig, with an increase in weight after thirty days, the grade was 4.0. These animals gained on the average less in weight than the others in this series, and correspondingly the average of hypertrophy was less. However, in the individual cases there was no definite parallelism between change in weight and degree of hypertrophy.

It is of special interest that even so large a dose as 0.1 gm. KI given daily does not necessarily prevent hypertrophy. It is possible that the very large dose is less favorable to the development of compensatory hypertrophy than the smaller doses; under similar conditions we found in the preceding series, in which KI was fed to the guinea pigs instead of being injected, the hypertrophy was less marked than in the animals receiving 0.01 and 0.05 gm. KI daily. Again the possibility exists that in these animals an excess of thy-

roxin was produced which counteracted to some extent the production of hypertrophy. However, the number of our experiments is as yet too small to allow a definite conclusion in this respect.

The structural characteristics in these gland remnants were similar to those found in the other groups of iodized guinea pigs, except that the proportion of acini which were smaller, with fewer phagocytes, a relatively lower epithelium and a harder colloid, was greater in this group.

If we compare the average degrees of hypertrophy in the control and iodized animals of the second series, we find the following figures: in control guinea pigs, 3.50; in guinea pigs injected with 0.01 or 0.05 gm. KI, 2.90; in guinea pigs injected with 0.1 gm. KI, 4.0.

The average degree of hypertrophy is, therefore, somewhat higher in the animals injected with 0.01 and 0.05 gm. KI daily than in the control animals, whereas a daily injection of 0.1 gm. KI was less favorable for the development of hypertrophy, although it did not prevent it.

Summary and Conclusions

1. Our new investigations concerning the action of KI in compensatory hypertrophy of the thyroid gland in the guinea pig confirm our previous conclusions, that administration of KI does not prevent hypertrophy, but that, on the contrary, the average degree of hypertrophy may be even higher in the animals to which as large a dose as 0.05 gm. KI has been administered daily than in the controls. In estimating the intensity of hypertrophy we must, however, take into consideration two facts which complicate the comparative rating of hypertrophy in iodized and control animals. (1) The change in weight of the animals during the course of hypertrophy is one of the variable factors which may affect the intensity of hypertrophy, and (2) secondary pressure effects, especially in iodized animals, may obscure the hypertrophic changes which had been previously established. Furthermore, removal of a great part of the thyroid gland as such acts as a stimulus to the gland, and the administration of iodine acting in this case as a superimposed stimulus, may be less effective than the administration of iodine to normal guinea pigs. Yet, notwithstanding these complicating factors, a consideration of all our series combined, in which iodine was administered to guinea pigs during the course of compensatory hypertrophy, makes it at least very probable that iodine itensifies rather than diminishes compensatory hypertrophy.

2. Our conclusions concerning the effects of iodine on compensatory hypertrophy hold good whether we leave behind only one-third to one-fifth of one lobe of thyroid gland as was done in our previous experiments, or one-half of one lobe as in our present experiments. Furthermore, we found that the effects are in principle the same, whether the iodine is fed by mouth or injected intraperitoneally.

3. An increase of the daily dose of KI to 0.1 gm. per day, corresponding to about 13 to 15 gm. of this substance in an adult person, although it did not prevent the establishment of compensatory hypertrophy, tended in our two series of experiments to diminish its intensity. Whether this result was due to a greater average loss in weight, produced through a non-specific action of so large an amount of KI in these animals, or whether conversely the loss of weight was due to an excess activity of the thyroid remnant called forth by so large a dose of KI, and an inhibiting effect of thyroxin, thus formed, on compensatory hypertrophy, must be left undecided at the present time.

4. The greater variegation in the structure of groups of acini in the KI fed animals, as compared with the controls, makes it in many cases possible to distinguish the compensatory hypertrophy of the thyroid gland, as it is found in the former, from the corresponding condition in the latter group of animals.

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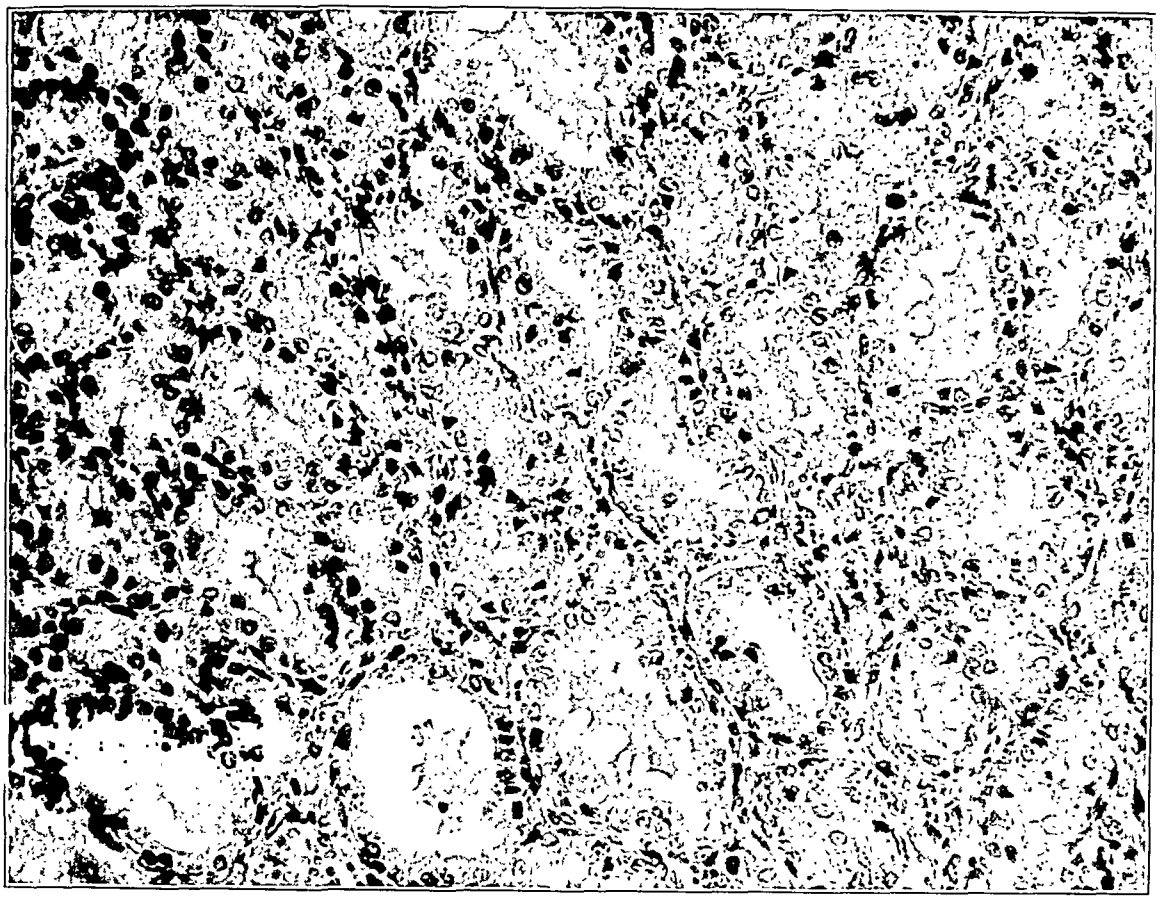
DESCRIPTION OF PLATE

PLATE 19

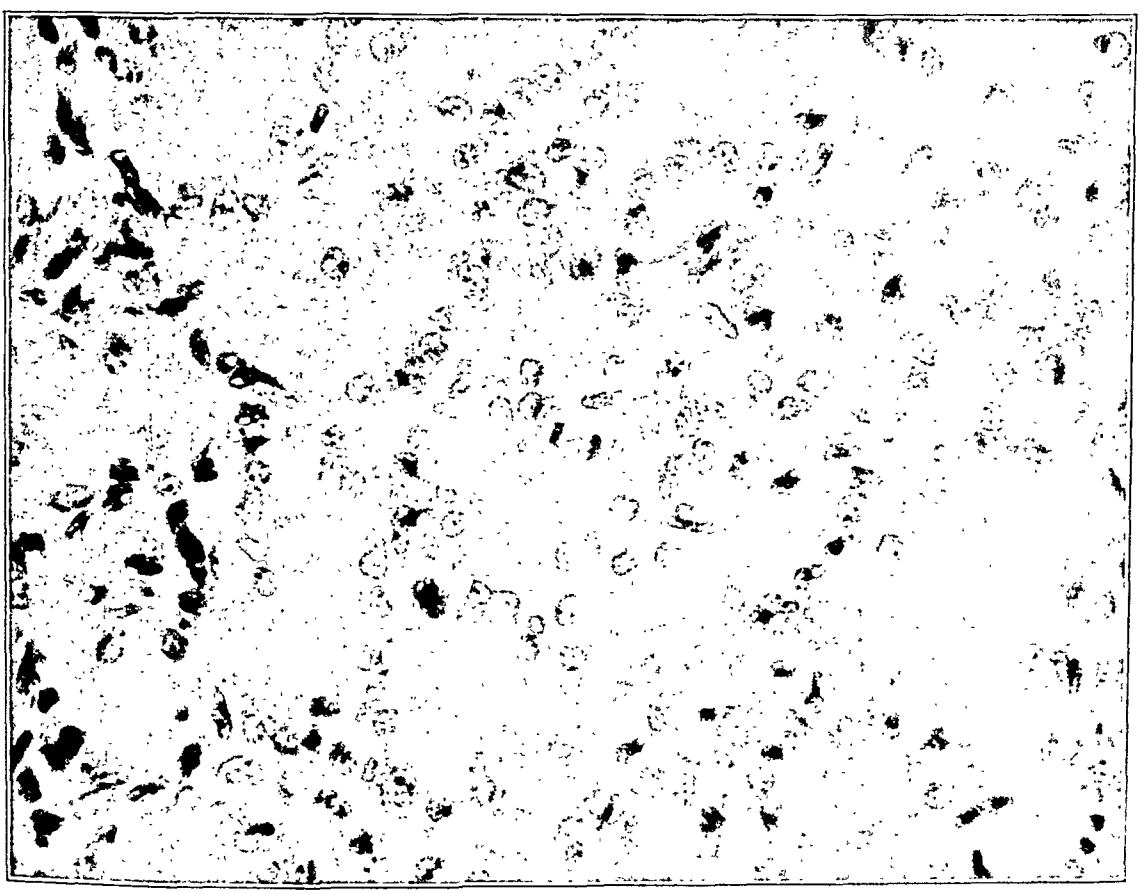
FIG. 1 and Fig. 2 are from Guinea Pig 945, male, weight 308 gm.; 1½ lobes of thyroid were extirpated on April 12, 1928; following the operation daily intraperitoneal injections of 0.05 gm. KI were given. Remaining part of thyroid taken out thirty days later, May 12, 1928; weight of guinea pig 365 gm.

FIG. 1 shows a part from the center of the remnant; there is marked hypertrophy of the acinus cells; the colloid is much diminished or lacking altogether. A number of the acini have been converted into slits. A mitosis is seen near the upper margin of the picture. × 300.

FIG. 2 shows a higher magnification of another part of the same piece. The colloid has entirely disappeared; the acinus cells are high. A mitosis is seen in the center of the picture. In the periphery of this piece the acini were much larger and distended by colloid which was in process of liquefaction. × 580.



1



2

Loeb Compensatory Hypertrophy of Thyroid Gland

THE EFFECT OF UNDERFEEDING ON THE PROLIFERATIVE ACTIVITY OF THE THYROID GLAND IN THE GUINEA PIG *

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In previous experiments Loeb [1] has observed that the degree of compensatory hypertrophy in the thyroid glands of guinea pigs is influenced by the changes in weight which the animals undergo during the period following partial removal of the glands. On the average the hypertophy was more marked in guinea pigs showing an increase in weight as compared with those in which the weight remained stationary or decreased, although in individual cases a loss in weight did not necessarily prevent hypertrophy. The change in weight constituted thus one of the variable factors which determined the growth processes in the thyroid gland. In accordance with these observations I found subsequently that the proliferation which occurs in the thyroid gland of normal guinea pigs under the influence of potassium iodide is on the average greater in animals which gain in weight during the course of the experiment than in those in which a decrease in weight takes place. These findings, together with other observations previously made in this laboratory [2] concerning the effect of undernourishment on the growth of the ovarian follicle and of the epidermis (to be published later), suggested the following investigation into the influence of undernourishment on the proliferative activity of the normal thyroid gland in guinea pigs. In regard to previous observations concerning the effect of inanition on the thyroid gland we may refer to the work of Jackson,[3, 4] and of Morgulis [5] in which the literature is fully discussed. Jackson made a very careful study of the thyroid gland in rats. In his experiments the period of underfeeding was much longer than in our investigations; he observed therefore much further advanced stages, especially marked degenerative processes. These are lacking in the guinea pigs studied by us. Furthermore our investigations differ from earlier ones inasmuch as we carried out a quantitative comparison of the

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proliferative activity in the underfed animals with the proliferative activity in the normal thyroid gland.

For this study twelve male guinea pigs of approximately the same weight were selected; five of them were underfed during the summer season, seven others were similarly treated during the winter months. This arrangement was considered advisable in order to obviate the

TABLE I

Underfed Guinea Pigs

No. of guinea pig	Original weight	Final weight	Loss of weight in grams	Per cent of loss of weight	Number of days during which underfeeding took place	Mitoses
904.....	365	248	117	32	11	0
903.....	380	275	105	27.6	11	0
902.....	395	280	115	29.1	11	120
905.....	400	290	110	27.5	11	0
400.....	380	295	85	22.4	10	0
401.....	390	275	115	29.5	10	0
402.....	405	290	115	28.4	10	0
403.....	380	300	80	21.	10	0
460.....	425	310	115	27.	9	0
461.....	410	325	85	20.7	9	0
462.....	395	295	100	25.3	9	0
463.....	405	300	105	25.9	9	0

influence of possible seasonal variations that might take place in thyroid activity. The underfeeding extended over a period of nine to eleven days; during this time the animals received the usual kind of food consisting of green vegetables and grain, but the quantity of food given each guinea pig was diminished in such a way that the animals subjected to this ration lost between 20 and 32 per cent of their original body weight. When this was accomplished the guinea pigs were killed with chloroform, the thyroids were at once removed and fixed and subsequently cut into complete serial sections. The number of mitoses in the entire gland were then estimated by means of the method described by us in a previous communication, according to which mitotic counts were made every tenth section.

The results we obtained may be briefly summarized as follows: the most striking feature in these experiments was the almost complete cessation of cell proliferation in practically every case (see Table I).

We thus found that out of the twelve underfed guinea pigs eleven failed to show any mitoses at all in the thyroid gland. In the one remaining animal we found the number of mitoses in the entire thyroid to be 120, which number is slightly below the average (see Table II).

We may therefore conclude that diminution in the amount of food intake has a very marked depressive effect on the proliferative activ-

TABLE II

Controls

No. of guinea pig	Original weight	Final weight	Gain of weight in grams	Per cent of gain of weight	Number of days during which gain in weight took place	Mitoses
259.....	368	385	17	4.6	10	150
265.....	318	348	30	9.4	10	440
337.....	470	525	55	11.7	10	264
342.....	495	570	75	15.1	10	170
Average of mitoses						256

ity of the thyroid epithelium, as evidenced by the absence of mitoses in the gland in almost all the animals examined.

As to the mechanism concerned in this process we may assume that the withdrawal of food from the animals lowers their metabolism in general as well as the metabolism of the thyroid gland, and in consequence of this effect the acinus cells cease to divide. The gland then enters a resting stage in which all activities come to a standstill.

The morphological character of the gland under these conditions confirms the conclusion that as a result of underfeeding its function is much diminished. In such a gland we find the acini reduced in size. These small acini are closely crowded together and thus the whole gland appears very cellular. The epithelium lining the acini is generally cuboidal and whereas in certain places it is flattened out, in other places it may be somewhat higher. The nuclei usually stain deeply with hematoxylin and appear granular. The colloid is very hard and fills the entire lumen of the acinus; the slight retraction of the colloid from the acinus cells which is usually found in a normal gland is lacking. Vacuole formation in the periphery of the colloid,

or other signs of liquefaction and absorption of the colloid is not usually observed in these glands. Furthermore, phagocytic activity which is so marked a feature in animals fed with KI, and which occurs likewise, although to a less extent in normal thyroids,[6] is practically absent in the colloid filling the acini of underfed guinea pigs. The structural condition of the gland corresponds therefore to that of a resting, not actively functioning gland.

In the light of these experimental findings we may conclude that the structural and proliferative conditions in the thyroid are influenced by the general body metabolism. Secondarily, the changes that take place in this gland must influence the metabolic energy. It is known that an increased thyroid activity is usually followed by an increased metabolism, and a decreased thyroid activity by a decreased metabolism. We now find that a diminished metabolism decreases the activity of the thyroid gland so that it approaches a resting stage. Under these conditions it is reasonable to assume that the reduction in general metabolism which occurs in case of general undernutrition is in part due to, or at least is intensified by the changes which take place in the thyroid gland as a result of underfeeding.

Summary

1. An experimental study was made to determine the effects of underfeeding on the thyroid activity in guinea pigs.

2. When young guinea pigs following underfeeding extending over a period of ten to eleven days lose from 20 to 32 per cent of their body weight, the acinar epithelium of the thyroid gland ceases to proliferate, as is evidenced by the entire absence of mitoses; the colloid becomes very solid and the acini small. The thyroid assumes therefore the appearance of a resting, not actively functioning gland. We may assume that this change in the character of the thyroid gland must still further reduce the general metabolism.

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THE EFFECT OF INTRAPERITONEAL INJECTION OF POTASSIUM IODIDE ON THE PROLIFERATIVE ACTIVITY OF THE THYROID GLAND IN GUINEA PIGS *

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In a previous communication [1] we have shown that the feeding of potassium iodide to normal guinea pigs for a period of two to three weeks results in a very pronounced increase in the mitotic proliferation of the thyroid epithelium, an increase which was even greater than that previously observed by Gray and Loeb.[2] Furthermore, we determined that there is a definite relation between the amount of KI fed and the increase in proliferative activity. Previously Loeb [3] had shown that feeding of KI to guinea pigs in which the greater part of the thyroid gland had been removed did not prevent compensatory hypertrophy, but on the average tended to increase the latter. Marine [4] on the other hand, maintains that the intraperitoneal injection of KI to guinea pigs previously subjected to partial thyroidectomy prevents the onset of compensatory hypertrophy. Since Marine did not feed KI to his guinea pigs but made use of intraperitoneal injections, it was thought possible that the differences in the results obtained by Marine and other investigators were due perhaps to the different methods of administration of iodide; we thought it therefore advisable to carry out another series of experiments in which, instead of feeding potassium iodide we injected it intraperitoneally. It was conceivable that the more rapid absorption of KI which takes place if the latter method is used might produce certain quantitative changes in the results obtained, and this possibility added to the interest of the experiment.

OUTLINE OF EXPERIMENTS

In this investigation four sets of guinea pigs were selected three of which were injected intraperitoneally with varying quantities of

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potassium iodide for different periods of time, while the fourth served as a control group. In the first set each animal received 0.01 gm. KI, in the second set 0.05 gm. KI, and in the third set 0.1 gm. KI, daily. The fourth set did not receive any potassium iodide. The potassium iodide to be injected was in each case dissolved in 0.5 cc. of sterile distilled water. This course of treatment was continued for periods of ten, fifteen, twenty and thirty days; at the end of each of these periods the thyroids were removed and studied in the manner described by us in our previous paper.[1] The weight of the guinea pigs used in these experiments varied between 350 and 500 gm.

Counts of Mitoses

Control Animals: The number of mitoses in the thyroid glands of the control animals varied between 136 and 280, the average being 212.

Animals Receiving Intraperitoneal Injections of KI for Ten Days: Animals that received intraperitoneal injections of KI for a period of ten days showed a considerable increase in the number of mitoses. In accordance with our previous findings this increase was greater, the greater the dose of KI administered. We thus found that the animal which received 0.01 gm. KI had 1050 mitoses, the one receiving 0.05 gm. KI, 2370 mitoses, while the guinea pig receiving 0.1 gm. had 4620 mitoses in the thyroid gland.

It is apparent from these figures that the intraperitoneal injection of KI for a period of ten days causes a marked proliferation of the thyroid epithelium, and furthermore that the larger the dose of iodide injected the more intense is the resulting mitotic cell proliferation. In our previous experiments in which the iodide had been given by mouth, the stimulating action of KI during the first ten days was very slight and it was not until the fifteenth or twentieth day that the marked stimulating influence of the substance was observed. It appears therefore that the route by which iodide is administered plays an important rôle in the rapidity with which the effects of this substance on the thyroid gland become noticeable, and that intraperitoneal injection acts more expeditiously than feeding by mouth. The more rapid absorption of the iodide when injected intraperitoneally is probably responsible for the effect at an earlier period.

Animals Injected with KI for a Period of Fifteen Days: In this group the number of mitoses in animals receiving 0.01, 0.05, and 0.1 gm. KI was 1040, 2828 and 2150 respectively. The counts were about the same as those obtained after ten days in the animals receiving 0.01 gm. KI; it was slightly increased after fifteen days in the one receiving 0.05 gm. KI, and was decidedly decreased in the last one receiving 0.1 gm. KI.

As compared with corresponding guinea pigs examined after ten days we may conclude that on the whole after fifteen days of KI injection the mitotic proliferation is still much increased over that found in the controls, but that it does not exceed the intensity found after ten days; it may even be somewhat lower. It may be suggested as probable that during the first ten days of the injection with KI the animal becomes more or less saturated with the substance and that in case of continued injection the response of the gland to the stimulating effects of the iodide becomes less.

Animals Injected with KI for a Period of Twenty Days: When the injection of KI is continued for twenty days there results a marked reduction in cell proliferation as compared with that of the shorter periods of iodide administration. The mitotic count however, is still higher than that found in the control group. Thus in animals receiving 0.01, 0.05, and 0.1 gm. KI the number of mitoses was 350, 470 and 840 respectively. In this period the response of the thyroid to the stimulating substance has become less.

Animals Injected with KI for a Period of Thirty Days: The number of mitoses in this group of cases was 520, 1120, and 1360 in animals receiving 0.01, 0 05 and 0.1 gm. KI respectively. We see then that there is still an increase in mitotic activity at this time over that noted in the control animals, and this increase is even somewhat greater than in the preceding period of twenty days. However, the difference between the counts found at twenty and thirty days is relatively small and further investigation must show whether it is a chance phenomenon, perhaps connected with the somewhat greater increase in weight of the animals of this group.

At present we can merely draw the conclusion that in animals injected intraperitoneally with KI for thirty days there is still an increased mitotic activity of the gland, whereas in our former investigations we have found that in guinea pigs fed with this substance for the same period of time all proliferation has ceased. Whether

this difference is due to differences in the mode of administration of KI used in these two series, or whether it is due to some secondary factor likewise needs further investigation. For a comparison of the number of mitoses found in the controls and in the injected animals at the various periods of time we refer to the table inserted below.

TABLE OF MITOSES *

Time in days	Control	0.01 gm. KI	0.05 gm. KI	0.1 gm. KI
10.....	264 (55+)	1050 (35+)	2370 (20+)	4620 (2−)
15.....	170 (75+)	1040 (130+)	2828 (100+)	2150 (5+)
20.....	136 (140+)	350 (98+)	470 (50+)	840 (5+)
30.....	280 (245+)	520 (115+)	1120 (130+)	1360 (38+)
Averages	212	740	1697	2242

* The figures in brackets indicate the gain or loss in body weight. + = gain in weight; − = loss in weight.

DISCUSSION

The question now arises as to the cause of the difference in effects on the thyroid of the oral administration of KI and of the intraperitoneal injection. The ready absorption of the iodide from the peritoneal cavity and its consequent earlier withdrawal from the blood by the thyroid might lead to a more rapid accumulation of this substance in the gland, the stimulation of the gland tissue resulting therefrom; also such a stimulation if continued for fifteen, twenty or thirty days, becomes less effective and the thyroid therefore reacts more sluggishly; thus the excessive proliferative activity is diminished.

The changes in proliferative activity of the thyroid gland of guinea pigs under the influence of intraperitoneal injection of potassium iodide, are represented in the curves of the attached chart in which in each case the number of days during which injections were given is the abscissa and the number of mitoses, the ordinate. If we compare this chart with the one in our preceding paper representing the variations in proliferative activity in the thyroid gland caused by oral administration of potassium iodide, we find some interesting differences. After feeding this substance the curves were relatively low during the first ten days but rose abruptly at fifteen days, reach-

ing the peak at the end of the twentieth day and falling again to normal, or even to subnormal levels at the end of the thirtieth day. After intraperitoneal injection of the iodide, the curves rise very

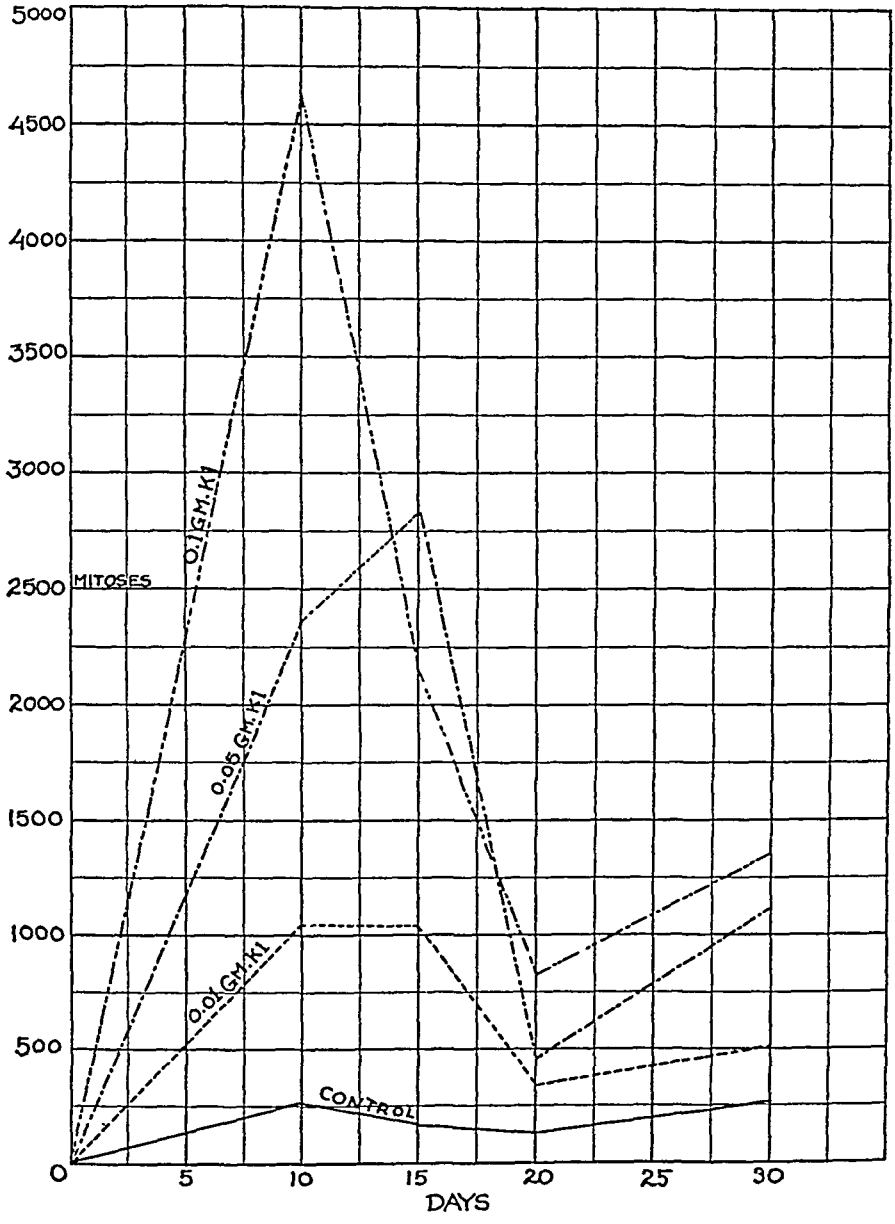


CHART I

much earlier and reach the peak at the end of the tenth day. From then on until the fifteenth day they either fall, hold their own, or show a slight rise. They all decline abruptly between the fifteenth

and twentieth day without however reaching the base line, and they remain still somewhat elevated after thirty days. There has even been a very slight rise between the twentieth and thirtieth day which may however be without significance.

In this series of experiments we find on the whole within the range of our experimental variations, that the increase in proliferative activity is the greater the larger the quantity of potassium iodide given. Only at fifteen days the proliferation is slightly less in the guinea pig receiving 0.1 gm. daily than in the guinea pig receiving 0.05 gm. of KI. We must however take into consideration the fact that the animals injected with the largest doses of KI did not gain in weight except the ones examined at thirty days when a slight gain was noticed, whereas the guinea pigs injected with the smaller doses all gained in weight. The daily intraperitoneal injection of as much as 0.1 gm. of KI into a guinea pig may influence the weight in this way by leading to an excess mobilization of thyroxin or by some other as yet undetermined process.

Structural Changes in Acini, Epithelium and Colloid: In the control series the acini are on the average of small or medium size lined with cuboidal epithelium and filled with solid colloid. We also find a moderate number of phagocytes in the colloid of some of the various acini. The microscopic appearance of the thyroid in animals injected with potassium iodide does not differ to any appreciable degree from that noted in the controls; the alveoli in the former are on the average of medium size, although they occasionally appear somewhat larger. The epithelium is cuboidal or columnar and the colloid is still solid but not to the same extent as in the control group. In the thyroid of animals injected with KI softening processes in the colloid are more marked, and correspondingly the number of phagocytes in the colloid is on the average greater than in the controls. However the watery, pale colloid that we noted in our previous experiments after thirty days of feeding of potassium iodide is entirely lacking in the injected guinea pigs. We also fail to find here the flattening of the lining epithelium and the consequent fusion and coalescence of neighboring acini which we noted at this period after oral administration of iodide.

Summary

1. The intraperitoneal injection of KI for a period of ten days causes a very rapid increase in proliferation of the thyroid epithelium, as evidenced by the great increase in the number of mitoses observed at this time. Between the tenth and fifteenth day the proliferative activity remains about equally high, except in the animal receiving the largest dose of KI where a decrease was noticed; it is considerably lower after twenty and thirty days of injection, but is still increased as compared with the number of mitoses found in the control animals.

2. In case of intraperitoneal injection of potassium iodide the average increase in proliferative activity in the thyroid gland is the greater the larger the amount of KI administered, within the range of the doses used by us. The curves representing the changes in proliferative activity of these animals, show, therefore, a characteristic difference from that representing the corresponding changes in guinea pigs fed with potassium iodide. These differences are due to differences in the rapidity of absorption and in the gradients of concentration of KI established between the blood and the thyroid gland and the organs through which elimination occurs.

3. The structural appearance of the thyroid of animals injected with KI does not differ very markedly from that of the controls. However, occasionally a slight softening of the colloid and a more active phagocytosis may be found in the injected animals.

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LYMPHOEPITHELIOMA *

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Lymphoepithelioma is a term employed by Regaud [1] and by Schmincke [2] in 1921 to designate certain tumors of mucous surfaces of the nasopharynx, which exhibit a specific structure and run a somewhat peculiar clinical course. Radiosensitivity was the main feature which led the observers to recognize in the tumors a peculiar clinical and pathological type.

The tumors occur at all ages, but are especially frequent between 30 and 60 years. They produce rather small local growths which are soft, slow to ulcerate, do not bleed much, and are therefore often overlooked and nearly always difficult to detect. The first symptom is often an enlargement of the cervical lymph nodes, multiple or bilateral, which may attain considerable dimensions before the primary tumor is detected. Distant metastases occur in the bones and in the liver, so that the course is usually fatal, although prolonged. The duration is generally about three years. The marked subsidence of the tumors under external radiation led the observers to conclude that the tumors were essentially different from the ordinary epidermoid carcinoma.

Support for this belief was obtained from the work of Jolly [3] and Mollier,[4] who have maintained that certain lymphoid structures in mucous membranes are covered with stratified epithelium which has a peculiar physiological relation to lymphocytes. Over Peyer's patches and the faucial and lingual tonsils, the epithelial layer is altered by the normal infiltration of lymphocytes. In the thymus the entire reticulum is of epithelial origin. The epithelial layer is

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generally thickened at the points where the lymphoid infiltration occurs. Intermediate grades of epithelial plication and lymphoid infiltration are observed in the bursa of Fabricius of birds, and in the pharyngeal tonsils. Hence Jolly designated these organs as lymphoepithelial.

In the human tumors derived from these organs the epithelial cells fail to assume squamous characters but remain undifferentiated, of rather large size, often forming syncytia, and are always accompanied by growth of lymphocytes. They may thus be difficult to distinguish from lymphosarcoma, but as a rule the epithelial elements are easily recognized as sheets of large flat cells.

The conception of lymphoepithelial organs, however, appears to be much older than Jolly's studies, 1914. Between 1886 and 1906 an active debate was carried on by Stöhr,[5] and others, and by Retterer,[6] the former holding that the lining epithelium of the tonsils is confined to the surface of the organ, while Retterer maintained that much of the reticulum of the tonsils is of epithelial nature and similar to the reticulum of the thymus. Jurisch[7] reviewed this subject at length. Retterer believed that in the tonsils and other superficial lymphoid structures there was the same intimate relation between epithelial reticulum and lymphocytes as in the thymus. At present, the evidence is against the participation of epithelium in the reticular structure of any lymphoid organ except the thymus, but it remains somewhat obscure why the thymus is the only lymphoid organ to enjoy this peculiar relation. It is also of interest to note that some tumors of the thymus present the most characteristic features of lymphoepithelioma.

The peculiar structural characters of certain epidermoid carcinomas of the nasopharynx have been noted by several observers, including Crowe and Baylor,[8] and New,[9] who reported several cases of this type and mentioned their resemblance to lymphosarcoma and the difficulty of finding the primary tumor. At the Memorial Hospital a group of radiosensitive nasopharyngeal tumors has long been recognized as peculiar in structure and as highly radiosensitive, and a series of such cases was reported by Quick and Cutler[10] under the term "transitional cell epidermoid carcinoma." This term was used because the grounds for assuming an origin from specialized epithelium as described by Regaud were regarded as not yet fully established. Their tumors arose from the tonsil, base of tongue and

nasopharynx, where stratified epithelium of transitional cell type is present.

A full exposition of lymphoepithelioma is furnished by Jovin,[11] who reports eight cases and discusses the theory of origin, the structure, and the clinical features. In his cases the tumor cells grew in wide sheets which were infiltrated with many lymphocytes (Figs. 3, 4, 5). The looser structure (Fig. 6), described by Schmincke and other observers, was not encountered. Six of the cases died from recurrences and usually with extensive metastases to the bones, but there were no autopsies. Two cases recovered after external radiation.

In Schmincke's cases the masses of epithelial cells were very loose and broken up by very rich admixture of lymphocytes, so that the structure resembled lymphosarcoma. Schmincke considered the question whether the tumors were not of mixed origin involving both epithelium and lymphocytes.

Derigs [12] reports a typical case arising in the pharyngeal wall of a subject of 15 years, with extensive metastases to bones, lungs and liver. The structure was very loose, the syncytial masses being broken up by many lymphocytes. She considered that the tumor process resided in the epithelium which, in all the metastases exerted a chemotactic attraction for lymphocytes.

Discussion

In a study of epithelial tumors of the tonsils and nasopharynx, the writer has been confronted with several questions which have considerable practical importance and the elucidation of which may have a bearing on the question of lymphoepithelioma.

1. Radiosensitivity is not an exclusive feature of lymphoepithelioma or of any one type of epidermoid carcinoma of the nasopharynx. Highly anaplastic carcinomas of tongue, tonsil and nares, which grow rapidly and metastasize widely, may melt down under moderate radiation. Very malignant tumors of this nature are also encountered in the uterus, esophagus, and pharynx. They produce relatively small primary growths, the metastases are very early and widespread, the cells are small, often rounded, resembling lymphosarcoma, and the response to radiation is very notable. In the group of cervical uterine growths, Cutler found a high proportion of three-year cures by radiation. It is possible that these tumors may be

distinguished from lymphoepithelioma by their more rapid course and by the absence of lymphocytes in the tumor tissue, but not by a difference in radiosensitivity.

2. Radiosensitivity may depend on embryonal qualities of the cells of origin, and the question arises whether any of the tumors of the nasopharynx arise from embryonal cells. The evidence in favor of this view is not very definite, but there is a well known group of tumors which may be called adenoid cystic epithelioma, which Krompecher classed with the basal cell group and which arise from the mucous glands of the nasopharynx. These may possibly be regarded as of embryonal type. They grow slowly and usually produce bulky tumors, but the metastases are late. They are moderately radiosensitive. The cells are small and appear in sheets inclosing mucous globules or larger cysts containing mucus. A resemblance to thyroid tissue is sometimes mentioned. They frequently recur after operation so that the eventual prognosis is serious. We have observed two such cases which recurred persistently after operation and radiation and eventually proved fatal, eleven and thirteen years after onset, and with very numerous metastases throughout the lungs and bone marrow. I think the metastases resulted from the extensive development of dilated veins about the tumor nodules into which the tumor cells forced a way. There is little difficulty in separating these growths from other types of epidermoid carcinoma and especially from lymphoepithelioma.

3. In the study of nasal carcinoma we have gradually reached the conclusion that the majority of them arise from the ordinary transitional epithelium which lines the Schneiderian membrane and the pharyngeal crypts and sinuses. This view appears to have been adopted by many observers, and the characters of Schneiderian carcinoma are well known. They are very cellular, growing at times diffusely, almost like lymphosarcoma, and the epithelial qualities of the cells are not prominent, or are entirely absent. The growth does not appear to be accompanied by infiltration of lymphocytes. There are doubtless many grades of malignancy with corresponding variations in the rate of growth but most of the tumors are recognized early because of ulceration and bleeding, and they progress steadily and often rapidly. It has often been noted that lymphatic metastases in this group are generally late.

4. The ducts of mucous glands at the base of the tongue and

throughout the pharyngeal wall are a possible source of transitional cell carcinoma, and there is considerable evidence that some tumors arise from this source. At the base of the tongue they may grow for some months before reaching the surface. Lymphatic metastases may therefore occur before the primary tumor is recognized, and in this respect they resemble lymphoepithelioma. In the metastases, epidermoid characters are usually distinct, but the growth may be very diffuse and it has proved impossible in our hands to distinguish some of these growths from the descriptions of lymphoepithelioma. The participation of lymphocytes in the primary or secondary tumors has not been observed; but bulky metastases in the liver or lungs have been a notable feature.

The radiosensitivity of Schneiderian and transitional cell carcinoma is very marked. The local growths may often be controlled by external radiation, but the outlook is unfavorable because of the metastases.

5. Carcinoma of the nasopharynx frequently occurs in young subjects between the ages of 10 and 20 years. The structure of these tumors resembles that of transitional cell carcinoma or lymphoepithelioma. The tumors grow slowly, but they eventually reveal their presence by nasal obstruction and bleeding. In many cases, however, the first sign of the disease is the swelling of cervical lymph nodes, but on account of the early age of the patients tuberculosis is usually suspected. In one case bulky metastases occurred in the liver, this organ being almostly completely replaced by tumor tissue (Figs. 7, 8).

The tumor cells are generally small, the nuclei relatively large and very hyperchromatic, and the cells form sheets or cords with comparatively little stroma reaction. I have seldom observed any definite admixture of lymphocytes. The distant metastases may be explained by the rich development of blood channels, some of which may be lined by tumor cells in immediate contact with the circulating blood.

The radiosensitivity is high, and after heavy external treatment considerable sloughing may occur. The outcome of many cases is unfavorable. I have been unable to trace the exact origin of the growths, but find that they occur at almost any point in the nasal passages or upper pharyngeal wall. It seems impossible to separate them from lymphoepithelioma or Schneiderian carcinoma.

6. In certain nasopharyngeal carcinomas one finds quite different structures in different parts of the same tumor, so that its classification becomes uncertain. I have observed two distinct structural types in the same carcinoma occurring in the nasopharynx of a young subject. In one area there were very small cells lining a blood sinus, while another area was composed of large flat cells approaching the squamous type. The occurrence of such cases suggests that the particular cell form of a tumor may not give a definite indication of its exact origin.

In a case of carcinoma of the frontal sinus there were areas of adenoma malignum, foci of adult cells approaching the squamous type, and small strands of small cells resembling transitional epithelium.

In the metastases the structure may be more anaplastic and undifferentiated, or less so. In a tumor of the base of the tongue showing a structure of transitional cell carcinoma or lymphoepithelioma, the structure in the cervical nodes was much more adult in type approaching squamous carcinoma in places, while bulky metastases in the liver were extremely cellular and undifferentiated. Since the metastases in lymph nodes are free from secondary changes due to infection and ulceration, the structure in the nodes should reflect more truly the original cell type.

7. The frequent occurrence of precocious metastases in cervical lymph nodes when a primary tumor of the nasopharynx exists, but long escapes detection, makes a heavy inroad into the field of so-called primary endothelioma of lymph nodes and renders the diagnosis of endothelioma of cervical lymph nodes a hazardous step. It is clear that a diagnosis of such a tumor in the neck cannot be accepted unless a long period elapses to permit the primary tumor to appear, or unless an autopsy proves the absence of such primary carcinoma. Moreover, the structure of certain transitional cell carcinomas or lymphoepitheliomas so closely resembles that which many authors including the writer have been in the habit of regarding as endothelioma, that the separation of the two groups of tumors on histological features must be regarded as extremely difficult, or often impossible. The mode of invasion of lymphoid tissue by certain lymphoepitheliomas is very peculiar, since it produces a structure which seems to spring directly from the lymphoid reticulum. There seems to be no way to determine whether these large

cells uniformly diffused through a lymphoid tissue are invading epithelium or multiplying reticulum cells. The chances favor a metastatic tumor in all such questionable cases.

Nevertheless, I continue to encounter occasional tumors of lymph nodes of the neck and other regions in which no primary tumor is discovered during a long period of the life of the patient or at autopsy, and which it seems necessary to regard as instances of primary endothelioma. The gross anatomy of the cases of endothelioma described by von Recklinghausen,[13] and others, especially those occurring in the abdominal region, is quite different from the conditions seen in metastatic carcinoma. There seems to be little doubt that primary endothelioma of lymph nodes does occur, but the scope of this group of tumors needs reconsideration in the light of the present knowledge of lymphoepithelioma.

The separation of cellular carcinoma of the tonsil from lymphosarcoma is also often difficult. Yet, in the epithelial tumors certain areas nearly always show sheets of cells of definite polyhedral form, while in lymphosarcoma the cells may be large but they never occur in sheets or with definite epithelioid characters. The gross anatomy and the clinical course of the two diseases are generally quite different.

Tumors of the tonsils and nasopharynx which may with reasonable accuracy be classed as lymphoepithelioma are comparatively rare, while tumors of transitional epithelium in which lymphocytes play no part are relatively common. As evidence on this point, I have reviewed 200 cases of epithelioma of the tonsils and base of tongue, and 100 cases of nasopharyngeal carcinoma, from the records of the Memorial Hospital, with the following result:

Tumors of tonsils and base of tongue	200 cases
Squamous epithelioma	72 per cent
Transitional cell carcinoma	12 " "
Lymphosarcoma	9 " "
Lymphoepithelioma	4 " "
Unclassified	3 " "
Nasopharyngeal tumors	100 cases
Squamous carcinoma	30 per cent
Transitional cell	37 " "
Lymphosarcoma	15 " "
Lymphoepithelioma	11 " "
Adenoma malignum	3 " "
Adenoid cystic epithelioma	4 " "

During this study it became apparent that there must be considerable personal equation entering into the decision as to which category certain tumors belong. Considerable difficulty was experienced in deciding on histological grounds whether the tumor was a lymphosarcoma or a carcinoma. In many cases clinical evidence seemed to be necessary to make this decision. The group classified as lymphoepithelioma did not appear to be very sharply defined, and it was at times difficult to decide whether to class the case with lymphoepithelioma, transitional cell carcinoma of Schneiderian origin, or as lymphosarcoma. The evidence furnished by several autopsies seemed inadequate to decide the question, since bulky metastases in the liver and bones occurred in all but lymphosarcomas.

I have had opportunity to study many sections of lymphoepithelioma with Dr. Laccasagne at the Radium Institute in Paris. I find that he includes under the term lymphoepithelioma, the various tumors which I have been designating as transitional cell carcinoma, and Schneiderian carcinoma.

Summary

The present available data seem to call for the recognition of a particular form of epidermoid carcinoma occurring especially in the nasopharynx and designated by Regaud and Schmincke as lymphoepithelioma. The tumors arise from modified epithelium overlying lymphoid structures in tonsils, base of tongue and nasopharynx. They occur at all ages, frequently in young subjects, grow slowly, are often overlooked, tend to produce early metastases in the neck, and later widespread extensions to liver and bone marrow, and are generally fatal. They exhibit a marked primary response to radiation. The diagnosis must be based on the structure, which shows sheets of pale staining epithelial cells, often in syncytia, and infiltrated with many lymphocytes, both in the primary tumor and in metastases. The diagnosis may not be based on clinical appearance, or course, or location, or radiosensitivity. Other tumors of the same regions are radiosensitive, produce early metastases to lymph nodes, bones, and viscera. Anaplastic carcinomas may be distinguished by their more rapid course and cellular structure.

The exact scope of lymphoepithelioma is not yet clear. Tumors of

transitional epithelium located in many areas of the nasopharynx and lining the ducts of mucous glands, seem to produce tumors showing many of the characteristics of lymphoepithelioma. It seems possible that the admixture of lymphocytes may be the result of a low grade inflammatory process occurring in certain other tumors, but their appearance in metastases is probably not to be explained in this way.

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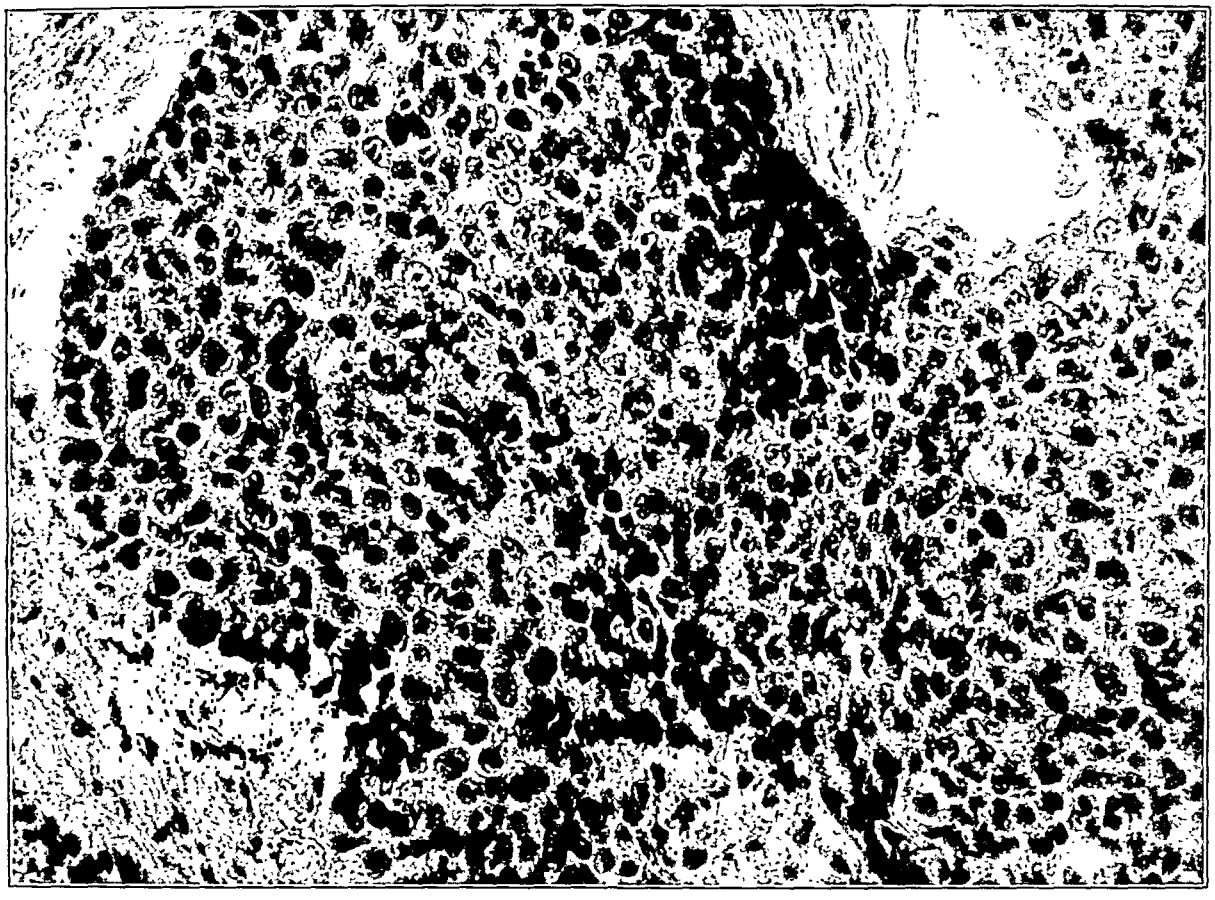
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DESCRIPTION OF PLATES

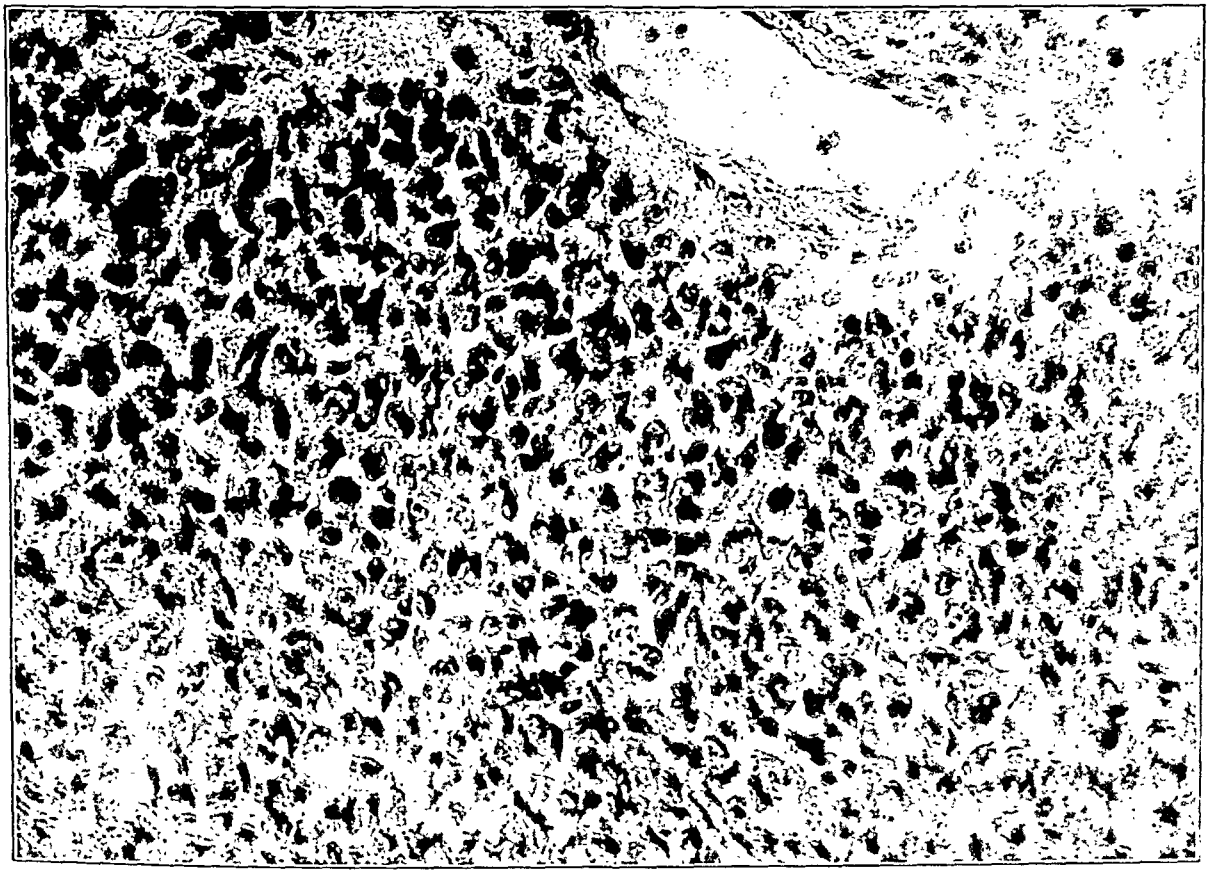
PLATE 20

FIG. 1. Epidermoid carcinoma of tonsil. Solid cords of coherent cells. Epithelial characters distinct. Transitional cell carcinoma.

FIG. 2. Epidermoid carcinoma of nasopharynx. Broad sheets of loosely coherent cells. Epithelial characters fairly distinct. Transitional cell carcinoma.



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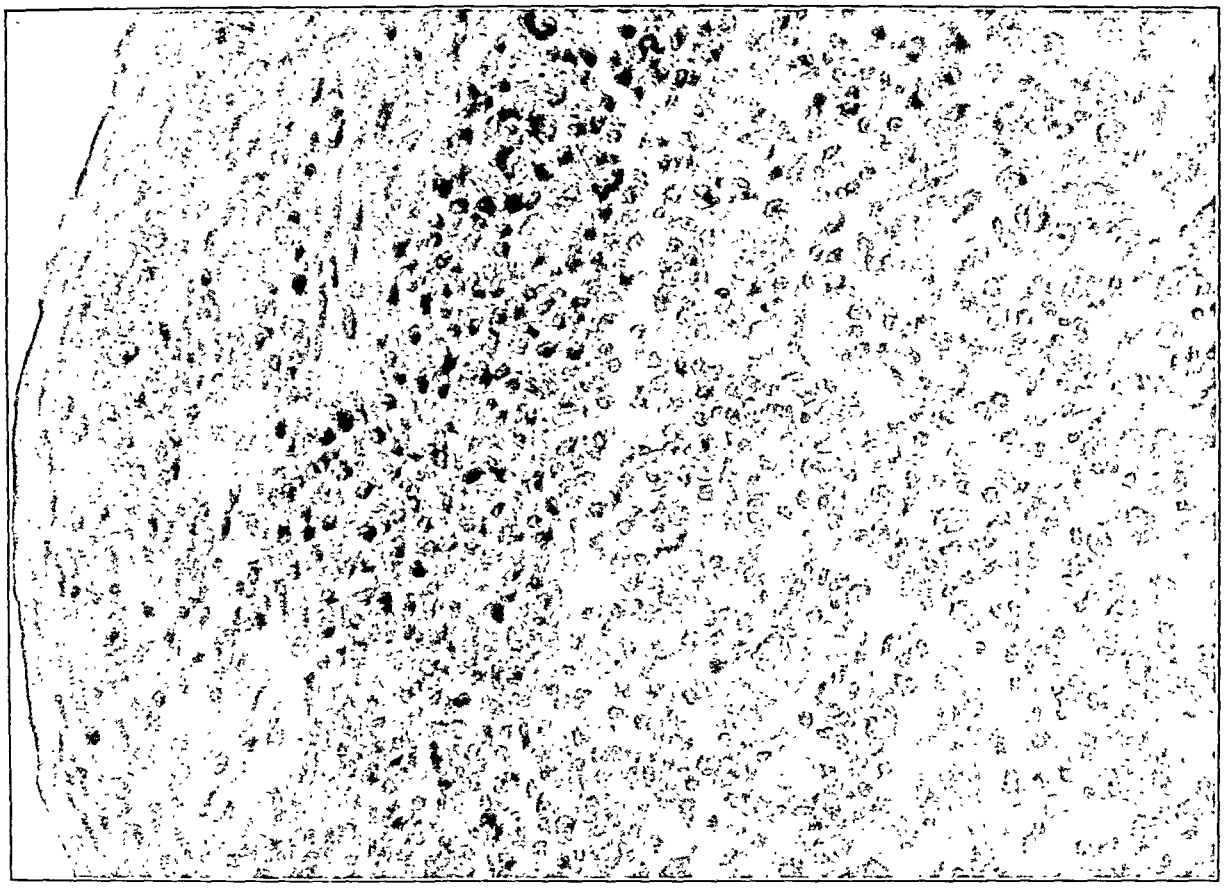
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Ewing Lymphoepithelioma

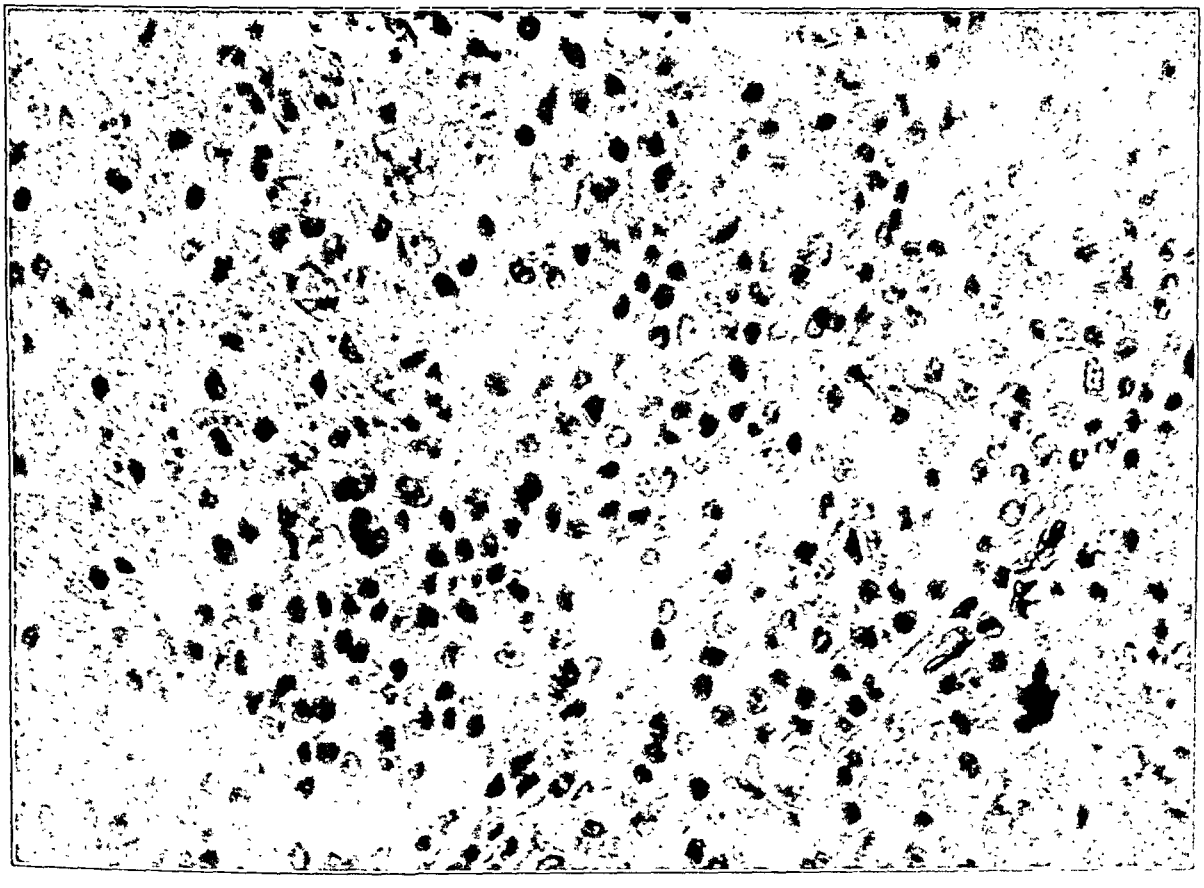
PLATE 21

FIG 3. Epidermoid carcinoma. Indistinct alveolar grouping of small polyhedral cells. Slight infiltration of lymphocytes.

FIG. 4. Epidermoid carcinoma of nares. Lymphoepithelioma of Regaud. Syncytial masses of large polyhedral cells with large nuclei. Rich infiltration by lymphocytes. Patient well three years after radiation.



3



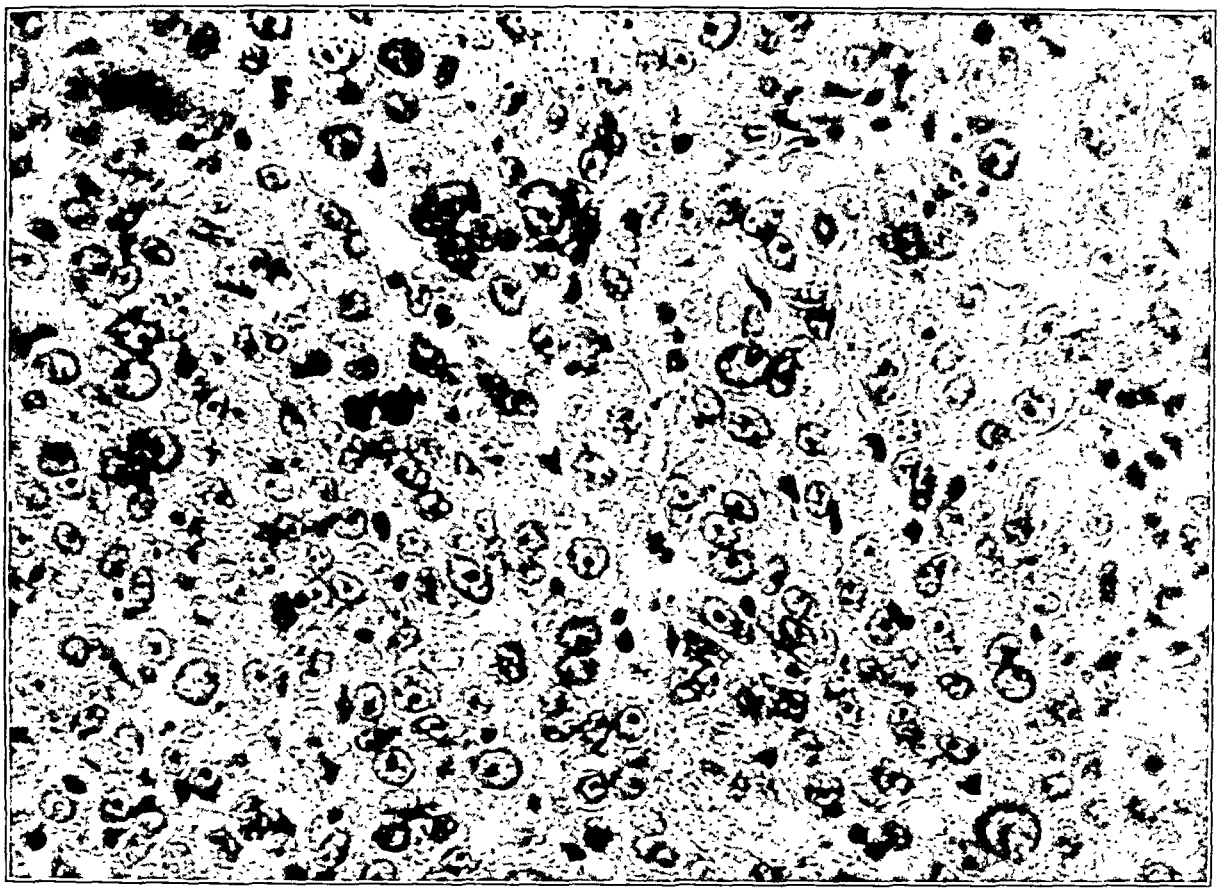
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Ewing Lymphoepithelioma

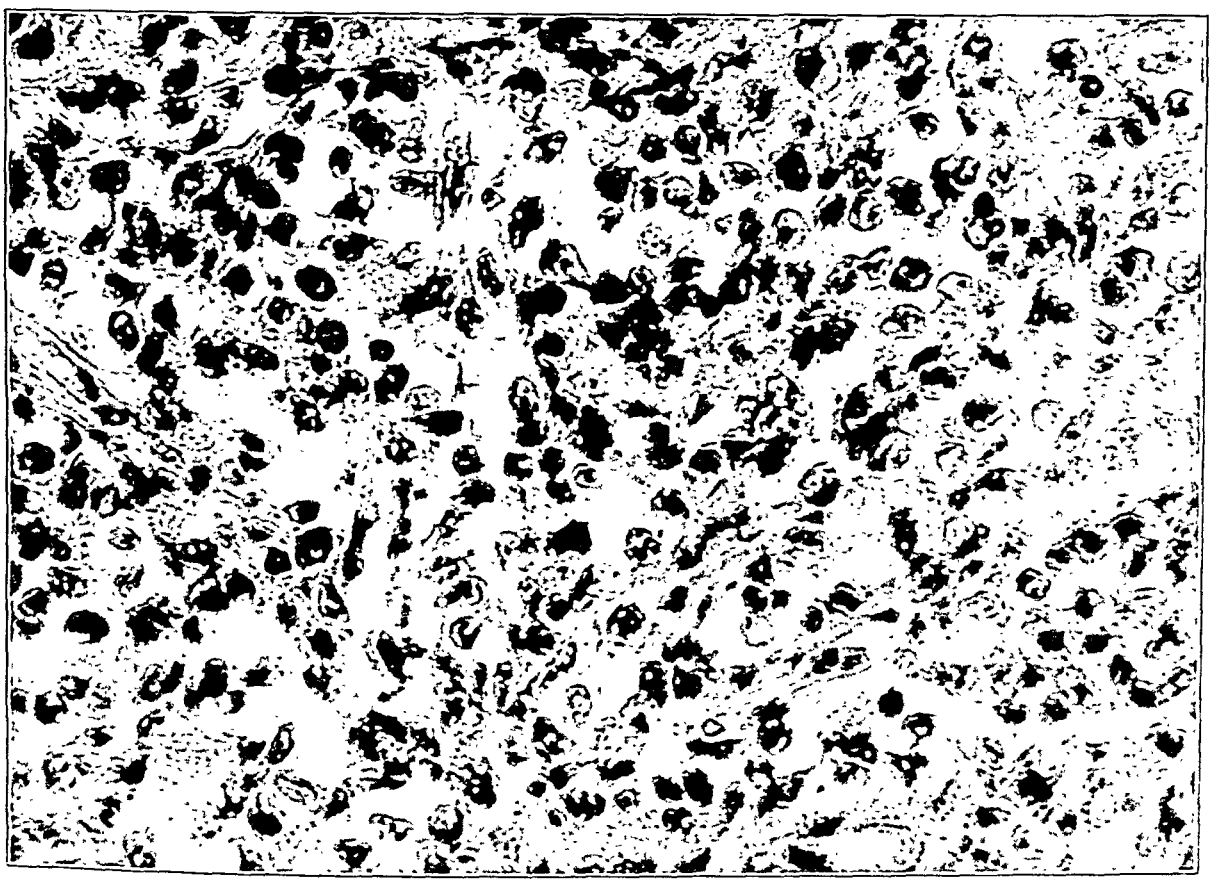
PLATE 22

FIG. 5. Epidermoid carcinoma of pharynx. Lymphoepithelioma of Regaud. Syncytial masses of large polyhedral cells. Infiltration by lymphocytes.

FIG. 6. Epidermoid carcinoma of nasopharynx. Lymphoepithelioma of Schmincke. Broad areas of loose hyperchromatic cells of indistinct epithelial type. The structure somewhat resembles lymphosarcoma.



5



6

Ewing

Lymphoepithelioma

PLATE 23

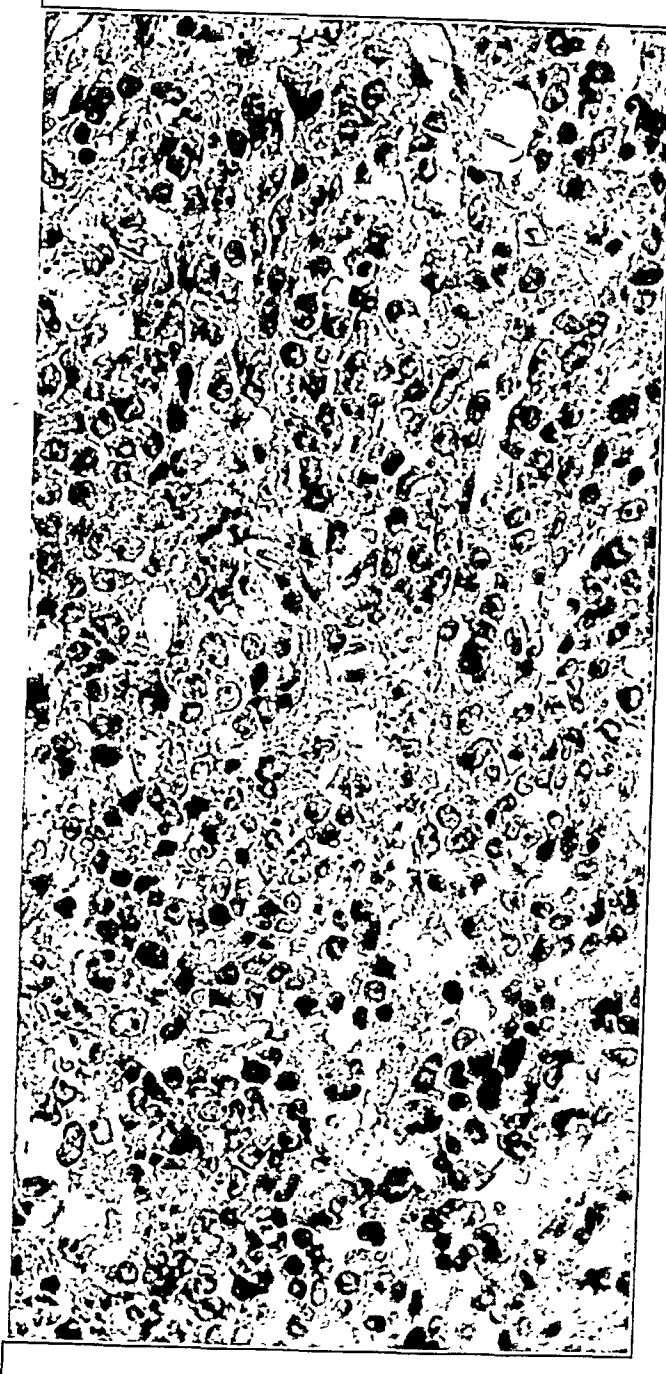
FIG. 7. Epidermoid carcinoma of nares in a subject 23 years of age. Lympho-epithelioma. Diffuse infiltration of entire liver.

FIG. 8. Epidermoid carcinoma of nares in a subject 23 years of age. Lympho-epithelioma. Structure of metastasis in liver.



7

Ewing



8

Lymphoepithelioma

BIOPSY HISTOLOGY IN THE GRADING OF RECTAL CARCINOMA *

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INTRODUCTION

In 1915 Broders began the practice of grading neoplasms on purely histological grounds and a series of studies [1, 2, 3, 4] by this author leaves very little question of the value of histologic grading of degree of malignancy in interpreting the probable course a given tumor type may be likely to pursue in a group of individuals. Like all purely laboratory methods it is apt to fail in the individual case wherein due and proper attention is not given to other influencing factors. A general review of the question of histologic prognosis of tumors is given by Plaut [5] and objections to the use of the method in individual cases in the absence of consideration of size and location of tumors, duration of disease and numerous other factors, are fully discussed. Apparently Plaut is not overimpressed with the value of the histologic prognosis alone in the individual patient.

Rankin and Broders [6] have recently applied Broders' method of tumor grading to a large series of rectal carcinomas. The material for grading came from 598 surgical excisions at the Mayo clinic. Upon the basis of histologic structure the tumors were grouped into four grades; the results would appear to be of distinct aid in estimating the probable operative result obtainable from the average patient.

We were interested in comparing the results obtained by Broders from a study of operative material, presumably affording opportunity for complete tumor examination and classification in accordance with the full pathological findings, with results obtained from examination of small, usually superficial, biopsy specimens. Since at the Memorial Hospital rectal carcinoma is but rarely excised, most of the cases falling within the inoperable group as judged by surgeons of the rectal clinic, almost no operative material is available for study. Furthermore the hospital has very little bed space avail-

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able for treatment of cancer in its terminal stages, and consequently but few autopsies have been obtainable. On the other hand some cases, while undergoing radiation therapy in the clinic, have had repeated biopsies thus affording a certain amount of material for the study of the question of change of tumor type during the course of the disease.

We have endeavored to determine as nearly as possible from our material the answers to the following questions: (1) the approximate number of tumors falling in each group; (2) the relation of age to tumor type; (3) the influence, if any, of site of tumor to histologic type; (4) the question of tumor type and duration of symptoms before the patient enters the clinic; (5) the duration of life from the onset of symptoms in cases of the various types dying of the disease; (6) metastases found at colostomy in each of the four groups; (7) change of tumor type as observed in repeated biopsies; (8) an attempted analysis of those cases in which marked discrepancy existed between the clinical course of the disease and the average clinical course which the disease should have run had it behaved in accordance with the tumor type.

Grading of Tumors

It is first desirable to explain, mainly photomicrographically, the grouping of the tumors. In general the grading we have employed approximates rather closely that of Broders and Rankin, but at the outset it must be stated that no very sharp lines can be drawn; what one sees is a series of gradual transformations in the direction of increasing anaplasia as the tumor grade changes from I to IV. There is room therefore for many individual variations in the grading and what we have sought are broad groups, necessarily with some overlapping in both directions, rather than hard and fast lines of division which cannot exist clinically.

Grade I: Under Grade I we have placed those tumors with malignant features, yet structurally approaching the benign adenoma. In tumors of this type the epithelial cells line distinct glandular cavities (Fig. 1). A papillary arrangement is frequently, almost uniformly present. Invasive tendencies, so far as determinable from the biopsy specimen, are negligible features. The epithelium contains two or three layers of nuclei, elongated, of rather uniform size,

without distinct hyperchromatism; mitoses are present but not numerous. The cell polarity is preserved, and the nuclei occupy the basal regions of the cells, leaving a cytoplasmic zone bordering the lumen of the gland. Secretory activity may or may not be present.

Grade II: In Grade II the tumor cells still line definite glandular cavities; these cavities are more irregular; there is a marked tendency toward the production of papillary ingrowths and outpocketings of varying sizes and shapes. The walls of the glandular cavities are thicker; nuclei are arranged in three or more layers; they are more hyperchromatic, larger, more elongated, and are situated through the entire thickness of the epithelium; no very distinct cytoplasmic border separates the nuclear layers from the gland lumina; evidences of ulceration and infection are fairly common; these tumors are classified as adenoma malignum or adenoma destruens, Grade II (Fig. 2). The beginning distribution of nuclei throughout the thickness of the gland wall may be interpreted as being an early expression of loss of cell polarity. Mitoses are often numerous.

Grade III: Tumors classified as Grade III (Figs. 3 and 4) show in addition to glandular cavities resembling those seen in Grade II, solid areas where glandular tendencies are either lost or are represented by abortive attempts at tubule formation; the cells may be small with hyperchromatic nuclei, or they may be quite large and spindle-shaped. In either case there is definite loss of cell polarity. Papillary characters are poorly marked or absent. The stroma is frequently markedly increased, whereas in the two preceding groups it is a less prominent feature. Often the strands of tumor cells present a marked elongated, drawn-out appearance suggestive of infiltrating tendencies. Ulceration and infection are frequent, and the feeling exists that when infection is marked the tumor grade for clinical purposes should be increased. Thus Figs. 5 and 6 typify tumors which ordinarily would have been given as Grade III but which were advanced in grade in view of the increased clinical malignancy resulting from infection. The clinical course of the two cases fully justified this advance in grading. Why infection should alter the clinical course in the direction of increased malignancy is not known; we do not know nor do we see any way of determining from our material whether the infection hastens the lethal termination by means of increased metastases or merely through the local and

constitutional effects of such infection, for example hemorrhage and toxemia.

Grade IV: Grade IV comprises a heterogeneous group of tumors; some of these suggest that with the accumulation of more material a fifth grade might profitably be introduced, but at the present time it is felt unwise to multiply grades. A classification under Grade IV has been given to certain tumors such as those illustrated in Figs. 7 to 10. These tumors show various structures ranging between adenocarcinoma and diffuse carcinoma; they may contain fairly uniformly small cells with small, round or spindle hyperchromatic nuclei, with an imperfect alveolar arrangement; they may consist of closely packed masses of tubulo-alveolar structures again with small cells, hyperchromatic nuclei and little stroma reaction. In the very diffuse type (Fig. 9) the carcinomatous structure may be determined with difficulty. The patient whose tumor is illustrated in Fig. 9 lived but five months from the onset of symptoms. Gelatinous adenocarcinomas characterized by diffuse infiltration with closely packed signet ring cells are likewise included in Grade IV. The above description indicates that the group as studied comprises a heterogeneous collection of tumors, no one of which has been met with sufficient frequency to estimate its prognosis as a single type.

Discussion

The distribution of tumors in the various grades was as follows: Grade I, 11 per cent; Grade II, 56 per cent; Grade III, 24 per cent; and Grade IV 9 per cent. The average age of incidence shows no marked difference in the various groups; it is slightly lower for Grade IV. The average age for Grades I, II, III is 58, 55, and 55 years respectively, whereas the average age for Grade IV is 47 years. It has been impossible to trace any relation between grade of tumor and location within the rectum. The average duration of symptoms previous to application for treatment of the rectal condition reveals certain grade differences; with Grade I it was twenty months, in Grades II and III, eleven and ten months, and in Grade IV, six months. Contrary to expectation no marked differences in average length of life were apparent in patients of the different age decades but with the same histological type of tumor.

Duration of Life: An attempt has been made to determine the length of life from the onset of symptoms to death with the four

tumor grades. This of course cannot be made with any marked degree of accuracy. Often the early symptoms are vague; a long history of hemorrhoids frequently confuses the picture and one is unable to decide when symptoms referable to some benign rectal condition give place to those of rectal cancer. The same objection of course applies to the analyses of age of incidence and duration of symptoms reported above. Duration cannot be accurately determined, but grade differences are sufficiently marked to merit attention. In cases dying, and presumably dying of the disease whether from metastases, hemorrhage, or infection, the duration of disease from onset to death was as follows: in Grade I, seventy months; in Grade II, thirty months; in Grade III, twenty-one months, and in Grade IV, ten months. In evaluating these groups, long operative survivals were eliminated; cases comprising the material had no surgical treatment other than colostomy. All had received more or less palliative radiation. The statistics offered are admittedly only approximations to the natural history of the disease.

Metastases: It is of interest to inquire what percentage of cases of the various grades had metastases; this again may not be answered with any degree of accuracy. For the determination of metastases we have only data derived from surgical examinations made at the time of colostomy. For this reason our figures show a considerably smaller number of cases with regional or distant metastases shortly after admission for treatment, than do those of Rankin and Broders. We admit the likelihood of their estimates. No case of Grade I tumor had metastasized at the time of colostomy; 10 per cent of Grade II tumors, 25 per cent of Grade III, and 60 per cent of Grade IV tumors had regional or distant metastases determinable at colostomy.

Change of Grade: The question of change in tumor grade during the course of the disease is of some interest. We have been able to study forty-six cases where more than one biopsy specimen has been obtained. Of these thirty-seven had two biopsies, eight had three, and one had four. In a total of 102 biopsies but ten grade changes were made; all but one of these consisted in an advance of the grade. In one case Grade II was changed to Grade I; in four cases Grade I was advanced to Grade II; in three instances Grade II was advanced to Grade III and in two cases the grade was altered from II to III and IV. It is safe to state that the tendency of a tumor of the rectum

is to maintain its type locally, rather than to develop progressively more malignant features. Again it must be emphasized that we are dealing with superficial biopsies and not the entire local tumor.

Prognosis: It is believed that the prognosis of rectal carcinomas with colloid or gelatinous features is somewhat better than in those cases where such characters are lacking. We have followed nineteen such cases, and have endeavored to grade the tumors from the appearance of the epithelial structures alone, neglecting the gelatinous elements. Unfortunately only in Grades III and IV may one draw any conclusion as yet. Of seven cases graded III, all are dead; the average duration of life from onset of symptoms to death was seventeen months. Of four cases in Grade IV, three are dead, the duration of the disease averaging thirteen months. The higher grades are restricted to those tumors containing large numbers of signet ring cells (Fig. 10). Despite this high mortality in the advanced grades certain operative results which we shall not attempt to summarize encourage the belief that the lower tumor grades of colloid carcinoma may run a very slow, relatively benign course, suggesting the value of surgery in this group even when tumors are recurrent.

Very little may be stated regarding those cases where marked discrepancy occurred between the estimated and the actual course of the disease. Almost all deaths took place outside of the hospital and the circumstances surrounding the deaths are not ascertainable. Local infection of tumors with resultant cachexia, hemorrhage; invasion of the bladder with resultant secondary urinary disturbances undoubtedly play a considerable rôle but occasionally one is at a loss to explain large differences between the estimated and the actual duration of disease. Almost all cases received radiation; as yet this factor's influence is not determinable.

Conclusions

1. The histological grading of rectal carcinomas in accordance with the suggestions of Rankin and Broders is of value in estimating the probable course of the disease.

2. Small biopsy specimens are sufficient for such grading.

3. Repeated biopsies do not show that change in type occurs sufficiently often in the course of the disease to interfere seriously with the prognosis suggested by the grade given at the time of the first biopsy.

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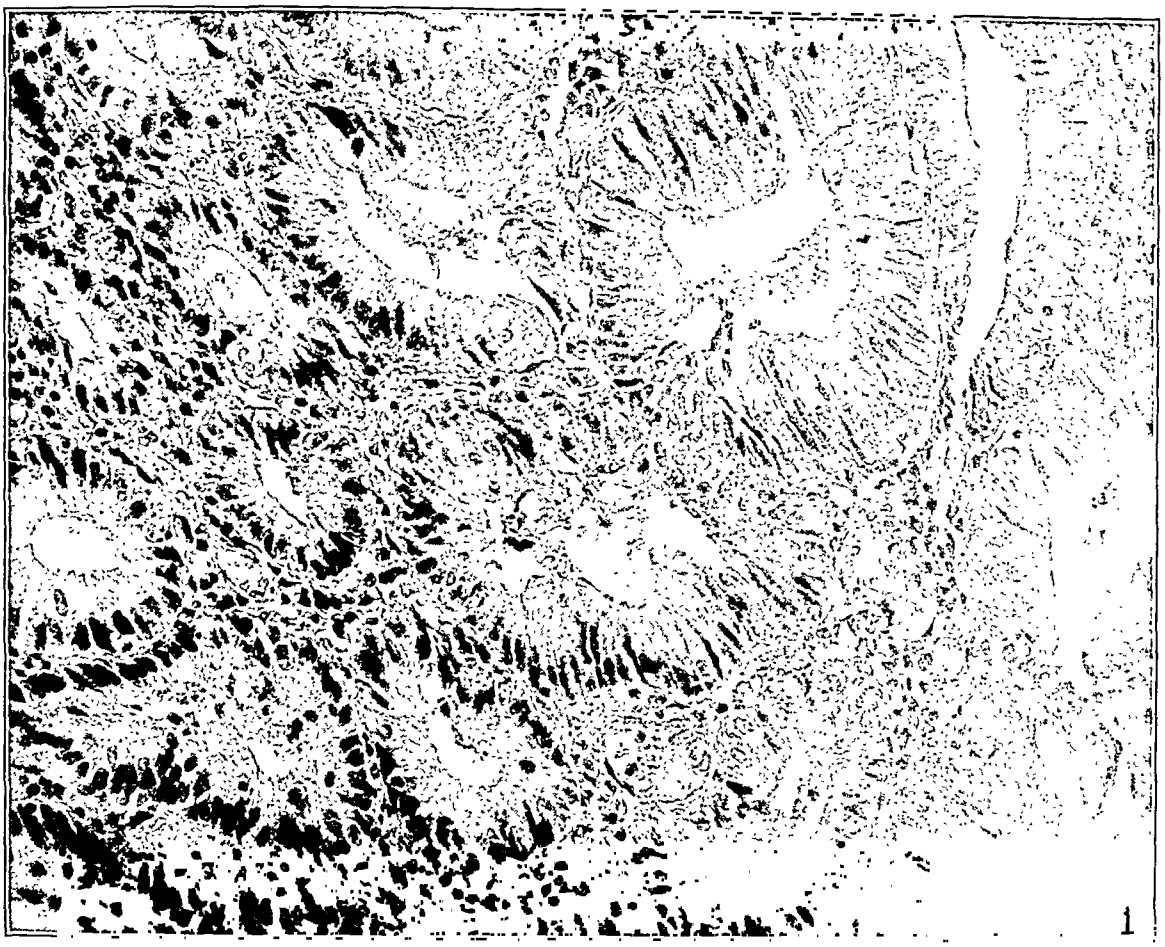
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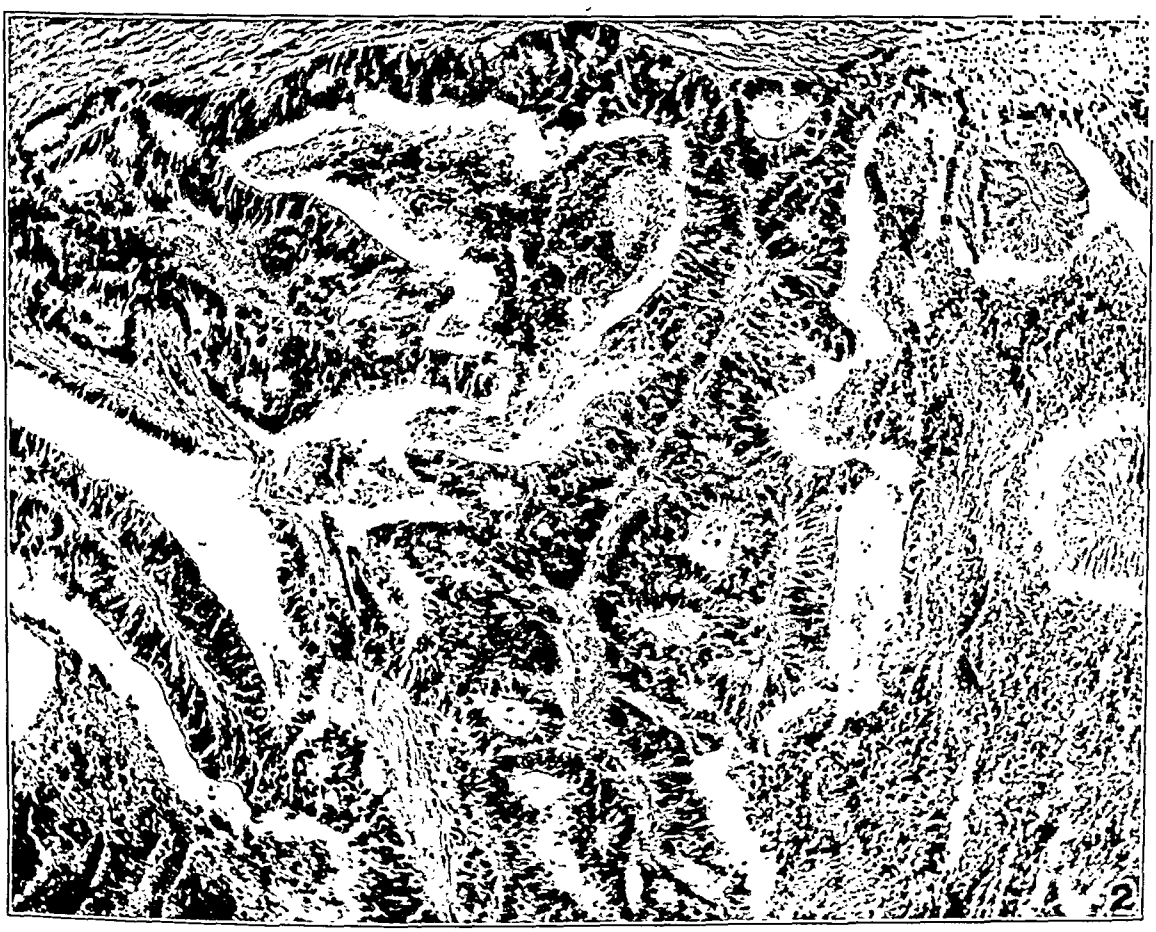
DESCRIPTION OF PLATES

PLATE 24

FIG. 1. Adenoma destruens, Grade I.

FIG. 2. Adenoma destruens, Grade II.



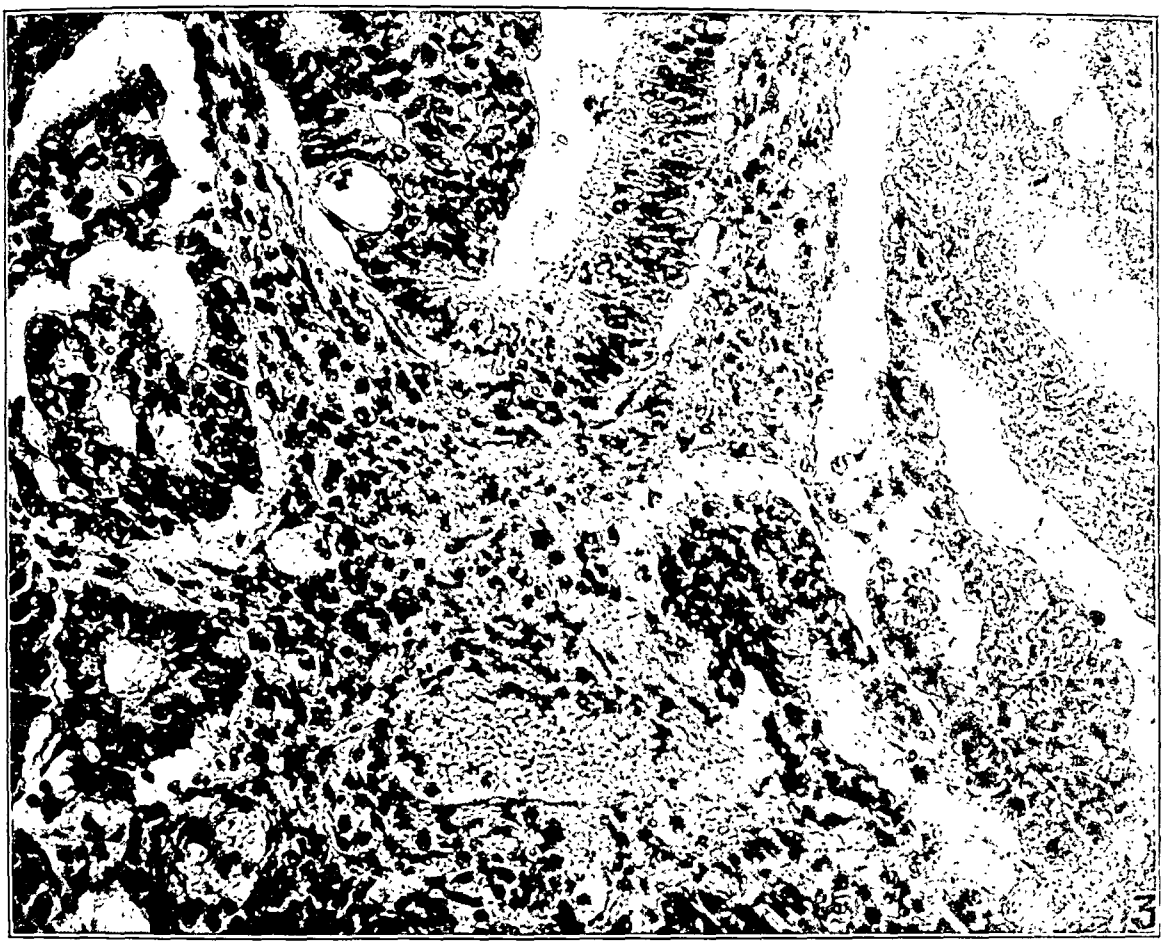


Stewart and Spies Histology in Grading of Rectal Carcinoma

Plate 25

Fig. 3. Adenocarcinoma, Grade III.

Fig. 4. Adenocarcinoma, Grade III.


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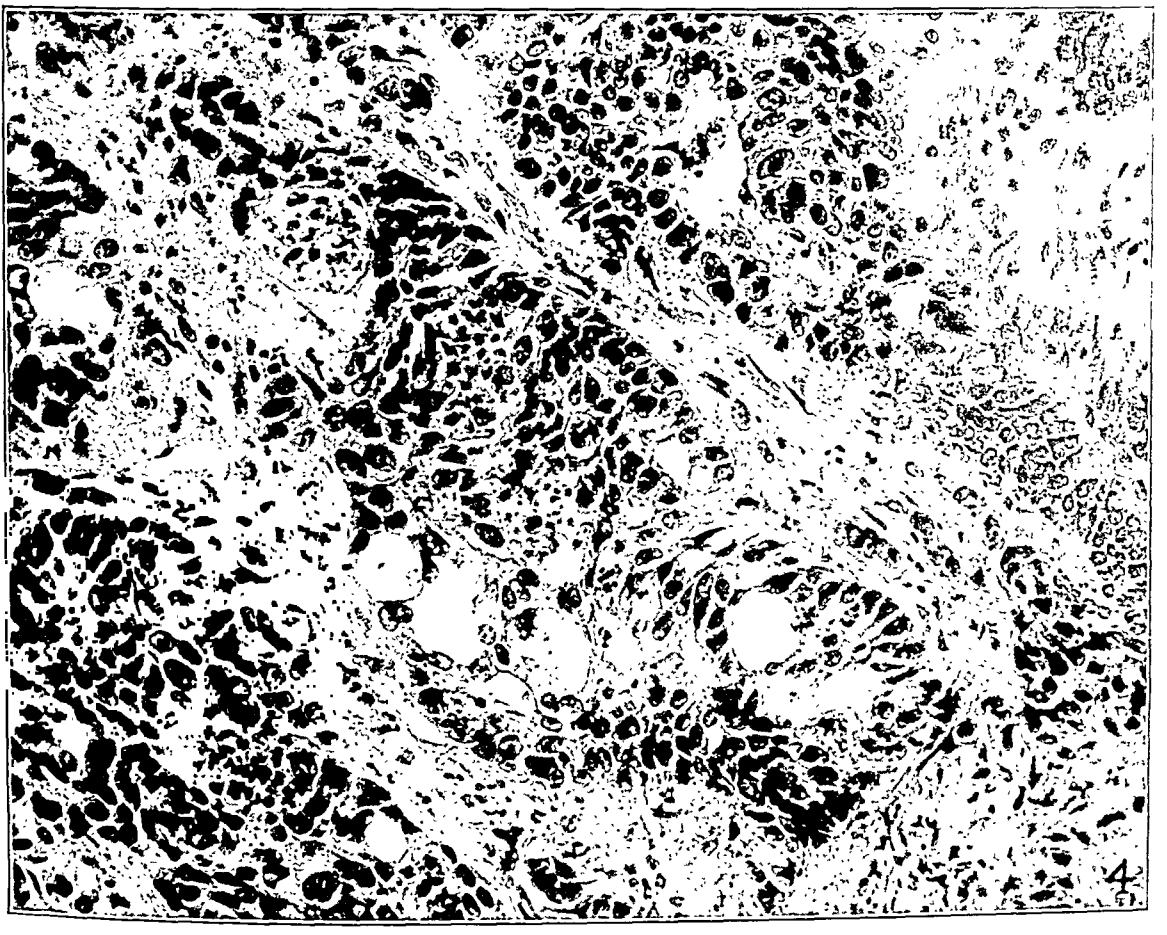
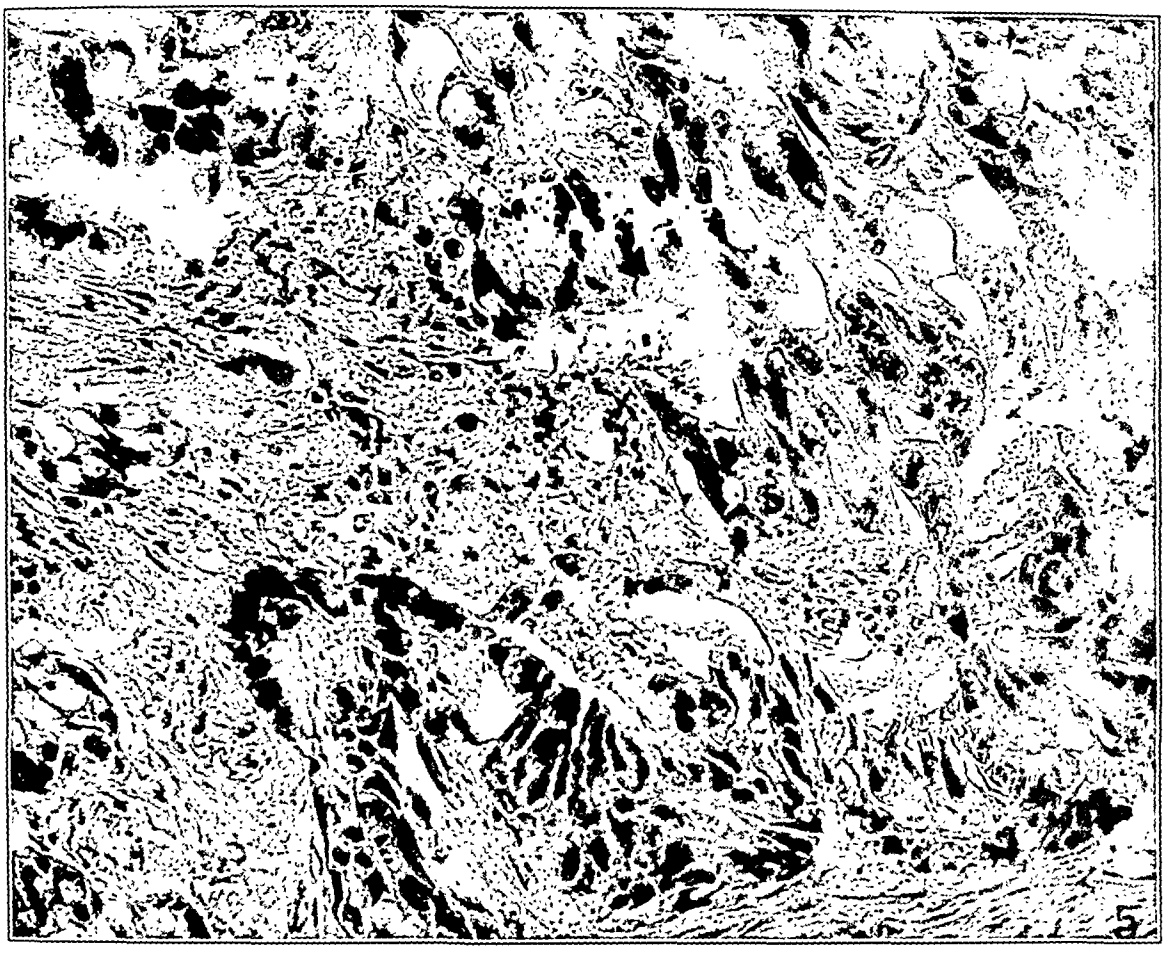
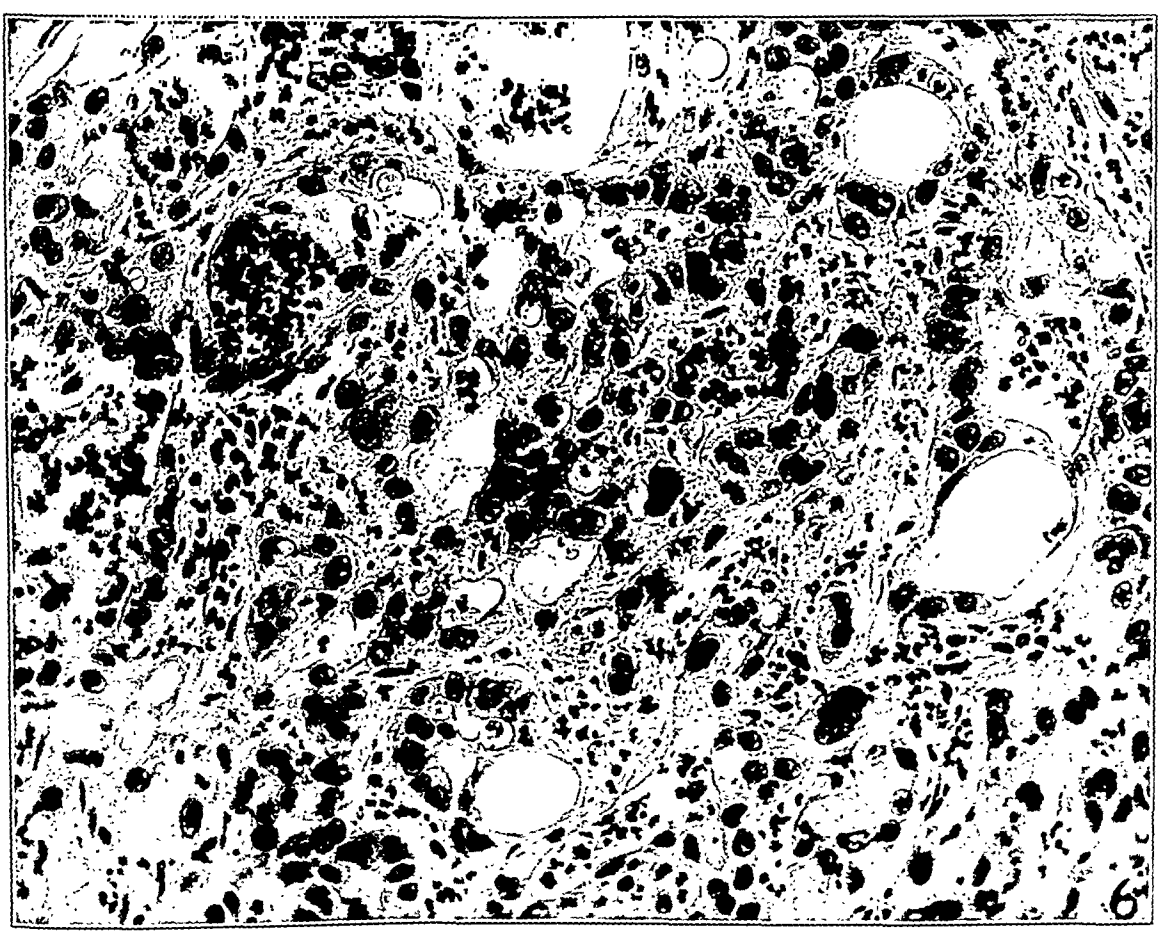

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PLATE 26

FIG. 5. Adenocarcinoma, Grade III, graded as IV on account of infection.

FIG. 6. Adenocarcinoma, Grade III, advanced to Grade IV on account of infection.



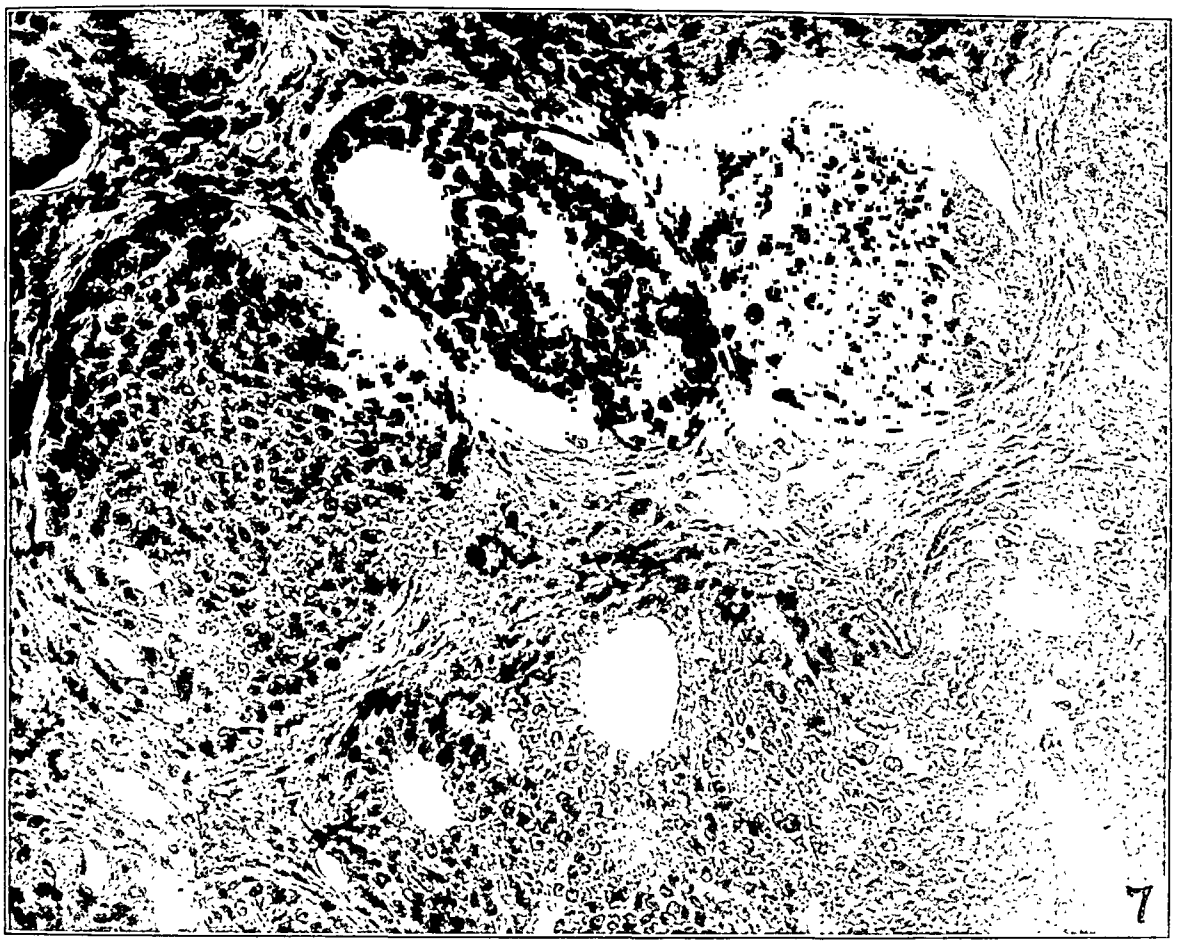


Steward and Spies Histology in Grading of Rectal Carcinoma

Plate 27

Fig. 7. Adenocarcinoma, Grade IV.

Fig. 8. Adenocarcinoma, Grade IV.


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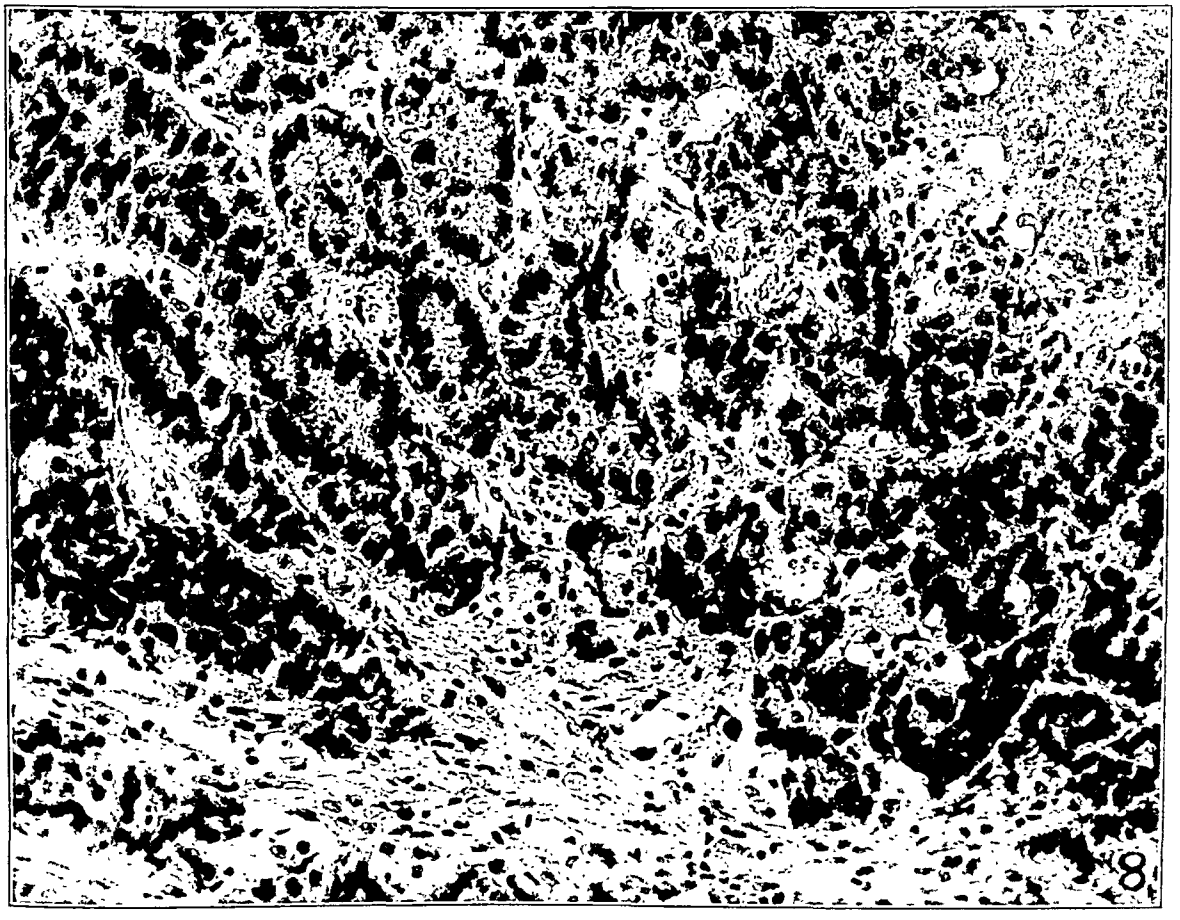
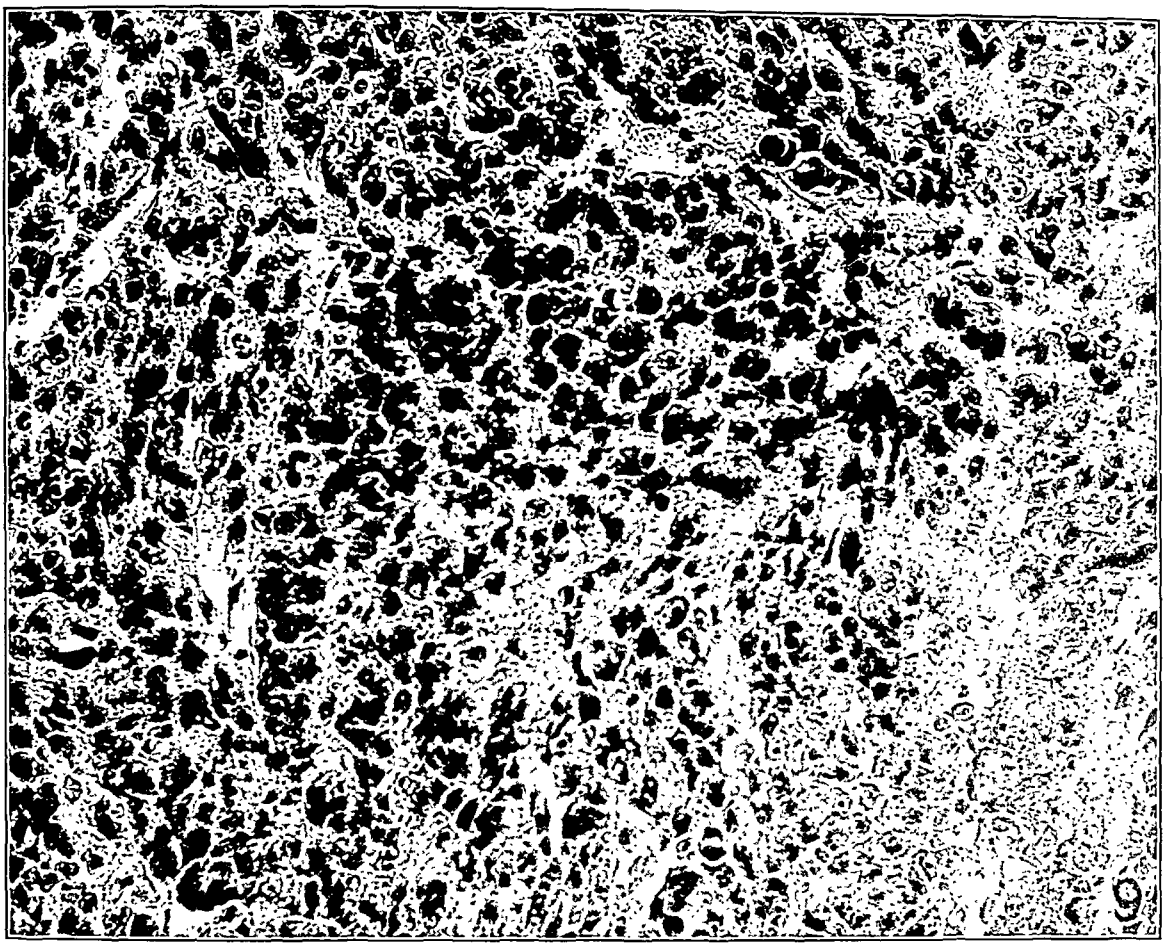
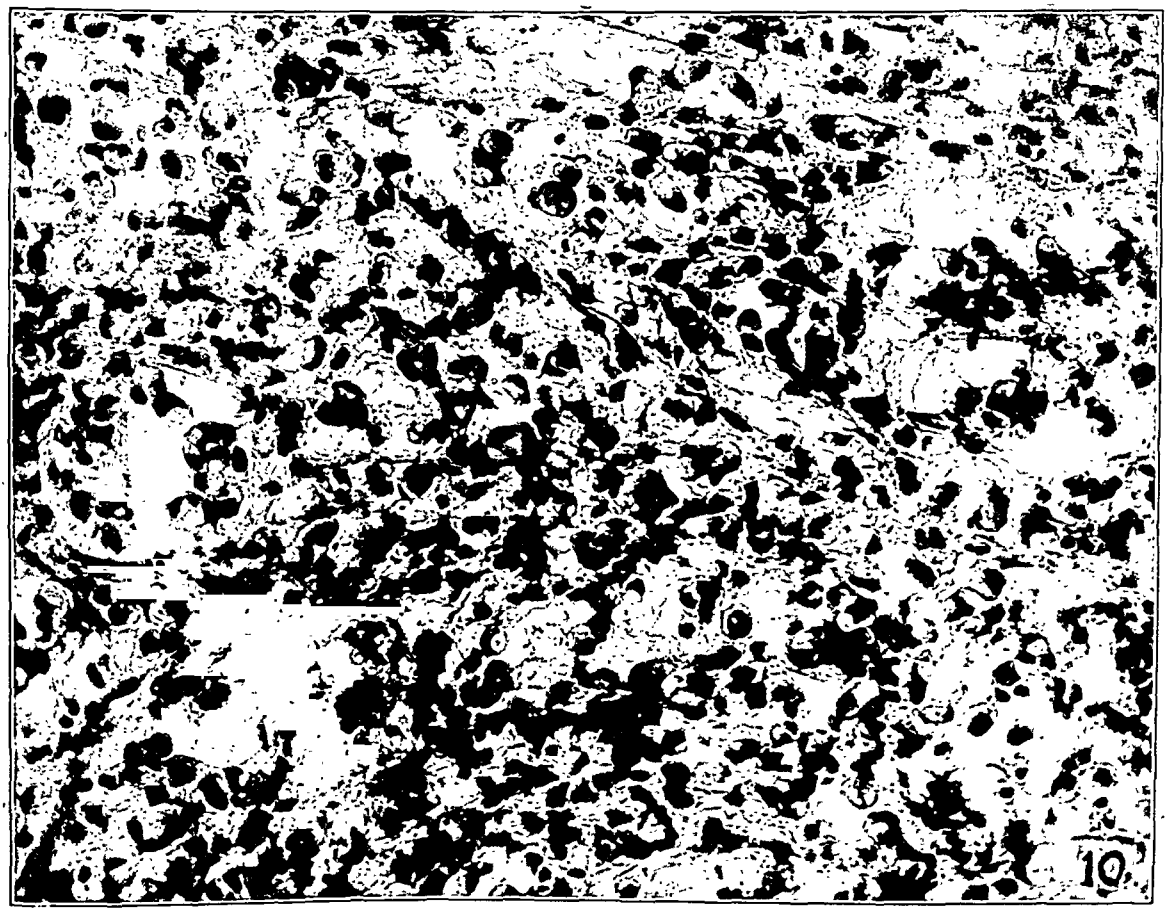

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PLATE 28

FIG. 9. Diffuse carcinoma, Grade IV.

FIG. 10. Gelatinous carcinoma with signet ring cells, Grade IV.





Stewart and Spies Histology in Grading of Rectal Carcinoma

THE PROGRESSIVE ANEMIA FOLLOWING A SINGLE INTRA-MARROW INJECTION OF B. WELCHII TOXINS *

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This study is in continuation of our investigation of the anemia produced in laboratory animals by a potent *B. welchii* toxin. In a previous communication attention has been directed to the consistently and abnormally high *B. welchii* counts in stools from pernicious anemia cases.[1] These findings have received confirmation in recent papers by Davidson,[2, 3] although Nye[4] believes that the difference from the normal as regards the number of *B. welchii* in the colon is more apparent than real. It was also shown [5] that a transitory anemia with a blood picture strongly suggestive of the pernicious type in a number of features could be produced in monkeys by intravenous inoculations of such a toxin. Similar observations for rabbits have also been made subsequently by Patterson and Kast,[6, 7] and Barach and Draper,[8, 9] although these investigators consider the resulting anemia of a secondary rather than primary type. The anemia produced by actual infection of rabbits with whole cultures of *B. welchii* was earlier studied by Cornell [10, 11] and by Reed, Orr and Burleigh,[12] and its pernicious-like features noted. It has been our experience, however, that the morphological and tinctorial blood changes in rabbits following intravenous inoculation of toxin, while somewhat suggestive of pernicious anemia, are not so striking as in monkeys. We have also reported [13] evidence of the absorption of this toxin from the gastro-intestinal tract of monkeys, the mucous membrane of which had been treated with a weak solution of sodium fluoride to induce a catarrhal inflammation. A blood picture in every way similar to that produced by intravenous inoculation resulted, together with a severe toxic degeneration of the marrow tissue, affecting particularly the fat cells and erythrocytes. The

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fluoride treatment alone caused no such marrow injury and only a moderate degree of anemia. The marrows of the two toxin-treated animals, however, were examined following their death from the anemia and there were thus no observations on the intermediate stages of the degenerative process. To gain information in regard to the effect of this sterile toxin on bone marrow, a large number of experiments have since been carried out in which a single small dose has been introduced directly into the tibial marrow of a series of rabbits and of a monkey. Somewhat to our surprise, we found that such a single treatment had a progressively injurious effect causing a chronic anemia with very marked loss of weight and frequently a fatal issue. Aside from any consideration of the possibility of absorption of *B. welchii* toxin being the essential etiological factor in the production of human pernicious anemia, the results aroused our interest because of the finding that the single inoculation into the marrow of one bone caused degenerative changes in quite equal, and at times in greater, degree in marrows of bones far removed therefrom. In view of the fact that the intravenous inoculation of the same dosage or a series of increasing amounts of toxin would not cause marrow changes of this character or degree, it is difficult to explain the apparent whole involvement of the marrow system following toxin injury to a single bone marrow.

It has been found that the *B. welchii* is capable of producing two apparently distinct hemolysins, one of which is active both *in vitro* and *in vivo* and the other, the more potent, causes blood destruction *in vivo* but only to a slight degree or not at all *in vitro*. Comparative tests of the action of these two products on bone marrow have been carried out both when introduced directly into the marrow and into the blood.

Technic

Toxin: The *B. welchii* toxin was prepared by the growth of the organism in casein-digest broth in sealed tubes as described heretofore.[2] We have found that 2 cc. of fresh rabbit blood per 30 cc. of medium may be substituted for fresh pigeon muscle. The toxin obtained is fully as potent and the *in vitro* hemolytic element is completely, or almost completely, absorbed during the growth period. This product will be designated as "toxin" in distinction from "hemolysin" which was obtained by growth of *B. welchii* in tubes

with the glass unsealed and in medium without addition of pigeon muscle or blood. Both products were rendered sterile by passage through Berkefeld filters. The toxin was tested for potency by injection in the breast muscle of pigeons. A good product caused death in 0.25 to 0.5 cc. dosage, in twenty-four hours.

Intramarrow Inoculation: The animal was placed under ether anesthesia, the hair shaved from a site over the outer posterior aspect of the left tibia about 2 cm. from the distal end and iodine applied. The skin was then stretched well over to one side and an incision, about 1 cm. in length, was made over the proposed point of inoculation. This was done so that when the skin was allowed to return to its normal position the puncture in the bone would be entirely covered and the danger of infection reduced to a minimum. The bone, almost directly under the skin, was laid bare and a small hole drilled of a diameter such that the hypodermic needle would fit snugly. This close fit is very important as otherwise the substance inoculated will not run into the marrow. The injection was made slowly, the usual dosage being 0.5 cc., and a few seconds allowed to elapse before the needle was removed. When properly carried out there was no leakage. With removal of the needle the skin was allowed to return to its normal position and a dressing applied. In no instance was there subsequently any evidence of infection and frequently when the animal was killed several weeks later the healing of the bone was so complete that the site of the puncture could not be found.

Experimental Results

The blood and marrow changes following the above treatment will be given first in detail in the case of a rabbit and a monkey and following that confirmatory tests will be described including a comparison of the action of *B. welchii* "hemolysin" and "toxin" introduced intravenously and into the marrow.

Rabbit No. 254: Male, weight 2200 gm. Inoculated into the left tibia with a single injection of 0.5 cc. of sterile "toxin" on March 11, 1926. The animal showed no obvious signs of discomfort following the injection. He was found dead seventy-five days later with a loss of weight of 580 gm., as shown in Table I. Twenty-four hours after the injection, both the hemoglobin and red cell count was reduced over 50 per cent. There was apparently also a marked leucocytosis

but this may have been a technical error as it was not noted in other rabbits similarly treated. About ten days following the inoculation the color index rose above 1.0 and stayed there until about two weeks before the death of the animal. For sixty days the animal was in a state of pronounced anemia with hemoglobin ranging from 50 to 60 and red cell count from 1,700,000 to 2,600,000. About one week before death a marked drop in hemoglobin occurred reaching a low point of 30. There was some evidence of a leucopenia, the white cell count reaching a minimum of 3,200 on two occasions. Morphological and staining changes in the blood were on the whole not quite so pronounced as reported heretofore as following intravenous toxin inoculations, although about five weeks after the toxin treatment poikilocytosis became very marked, much more so than when the toxin was injected intravenously. Nucleated red cells, punctated basophiles or those containing Howell-Jolly bodies were seldom observed. Pronounced anisocytosis was noted twenty-four hours following the injection and by the fourth day the microscopic fields showed principally microcytes staining more bluish with the Wright stain than normally. At least three attempts at regeneration are discernible; on March 19, April 10 and May 10. At these times there was a slight rise in hemoglobin or erythrocyte count and the blood picture was more nearly normal. On the day before death there was very marked anisocytosis, poikilocytosis, achromia and polychromatophilia. Two nucleated erythrocytes, three myelocytes and one myeloblast were found per 100 leucocytes counted.

For all the reports on the microscopic findings in the marrows and other tissues in this and other animals covered in this study, we are one indebted to Dr. James Ewing.

The marrows of the left and right tibia, right femur and left humerus were a dark maroon color throughout in the case of the two latter, and of a lighter shade in the distal halves of the two tibias. Sections of marrows from the left and right tibias, the left femur and left humerus showed essentially the same changes. Part of this tissue had been fixed in 10 per cent formalin and part in Zenker's fluid.* Dr. Ewing's report was as follows:

"There is in all a replacement of fat and lymphoid cells by a peculiar homogeneous granular material which appears to replace

* Our appreciative thanks are due to Dr. W. B. Castle of Boston City Hospital, who had sections of some of the Zenker-fixed material prepared.

TABLE I

Single Inoculation of B. Welchii Toxin into the Bone Marrow of a Rabbit

Rabbit No 254. Mature, Male. Weight 2200 gm.

Date	Toxin inoculation	Hemoglobin	Color index	Red blood count	White blood count	Differential white cell count							Anisocytosis	Poikilocytosis	Nucleated reds	Punctated basophiles	Howell Jolly bodies
						Polymorphonuclears	Lymphocytes	Basophiles	Large mononuclears	Eosinophiles	Transitional	Myelocytes					
1926		*per cent*															
Mar. 11	Normal	82		4,310,000	7,000	2	75	–	2	20	1	–	–	–	–	–	–
" 12	0.5 cc.	40	1.0	2,080,000	24,000	3	77	–	1	20	–	–	+++	–	1*	++	–
" 15	*B. welchii*	50	0.7	3,080,000	5,800	1	89	–	2	8	–	–	++++	–	–	–	–
" 19	toxin into	62	1.1	2,690,000	6,900	3	86	–	1	11	–	–	++	–	–	–	–
" 22	tibial	60	1.4	2,130,000	5,000	2	85	–	4	9	–	2	++	–	–	–	–
" 25	marrow on	55	1.4	1,910,000	3,200	–	72	–	1	26	1	–	+++	–	–	–	–
" 30	Mar. 11	51	1.4	2,150,000	5,200	–	78	–	–	21	1	–	–	–	–	–	–
Apr. 3		59	1.1	2,630,000	3,200	–	84	–	1	13	2	–	+++	–	–	–	–
" 10		61	1.4	2,100,000	5,000	–	73	–	1	25	1	–	–	–	–	–	–
" 17		58	1.3	2,240,000	2,400	4	72	3	–	21	3	1	–	++++	–	–	–
" 26		57	1.7	1,770,000	4,200	–	72	–	2	25	1	–	++++	++++	–	–	++
May 3		50	1.3	1,860,000	3,200	–	60	–	4	35	1	1	++++	++++	–	–	++
" 10		56	1.0	2,770,000	4,600	–	78	–	3	19	–	2	+++	+++	–	–	–
" 17		35	0.8	2,080,000	5,800	–	–	–	–	–	–	–	–	–	–	–	–
" 24		30	1.0	1,520,000	2,400	–	81	–	2	17	–	3	++++	++++	2	–	–

May 25 Rabbit died. Weight 1620 gm. at death.

* Per 100 leucocytes.

these cells and compose four-fifths of the tissue. The few remaining cells are mostly rather large, hyperchromatic granular mononuclears of the myelocyte type. No definite traces of forming red cells could be found. The changes resemble the so-called myeloblastic degeneration of bone marrow in pernicious anemia in an advanced atrophic stage." These findings are illustrated in Figs. 1 and 2. It may be noted that the degree of degeneration is even more marked in the right tibial marrow than in the left, into which the toxin had been injected.

Monkey Q: Mature, Nicaraguan Bull Head, Cebus type, female. May 6, 1926, 0.5 cc., *B. welchii* toxin inoculated into right tibia. A progressive anemia developed. When killed seventy-seven days later the animal was very anemic. The erythrocyte count was 1,700,000, the hemoglobin, 40. The tongue was pale and smooth, the liver, a light yellow reddish brown, the spleen, not enlarged and a light pink. No obvious lesions in the kidneys. The intestine was practically free of parasitic worms. The bone marrows were a dark maroon throughout, with the exception of those of the tibias in which the distal ends were of a lighter color. In this animal and in all *B. welchii* inoculated rabbits the blood serum was either colorless or a light straw color and never showed the lemon yellow hue so characteristic of pernicious anemia. The details of the blood examinations are shown in Table II. It will be noted that the erythrocytes fluctuated around 2,000,000 with counts below that mark during the last three weeks and the hemoglobin between 40 and 60 with evidences now and then of attempts at regeneration. The color index was generally slightly above 1.0 and there was at times a moderate leucopenia. Marked anisocytosis with many macrocytes was noted at practically every examination with occasionally distinct poikilocytosis and polychromatophilia. Occasional nucleated reds and myeloblasts were seen and Howell-Jolly bodies were considerably more prominent than in the blood of the rabbits similarly treated. In general features, however, the findings were very similar to those for the rabbits.

Microscopic examination of marrows of the right and left tibias, a femur and humerus showed degenerative changes, perhaps more marked in the right tibia into which the toxin had been inoculated, but prominent in the other marrows. The right tibia showed very numerous fat cells with scanty marrow cells which lay in clusters

TABLE II

Single Inoculation of B. Welchii Toxin into the Bone Marrow of a Monkey

Monkey Q. Young Adult Female

Date	Toxin inoculation	Hemoglobin	Color index	Red blood count	White blood count	Differential white count							Anisocytosis	Poikilocytosis	Nucleated reds	Punctated basophiles	Howell Jolly bodies
						Polymorphonuclears	Lymphocytes	Basophiles	Large mononuclears	Eosinophiles	Transitional	Myelocytes					
1926		*per cent*															
May 5	Normal	78		4,250,000	8,800	57	36	–	2	3	2	–	–	–	–	–	–
" 6	Normal	74		4,780,000	9,600	62	32	–	2	3	1	–	–	–	–	–	–
" 6	0.5 cc.																
" 10	B. welchii	42	1.05	2,030,000	5,000	26	69	–	–	3	2	++	+	–	1*	–	–
" 17	toxin in	45	0.9	2,320,000	5,000	37	59	–	1	1	2	–	++++	++++	–	–	–
" 24	tibia on	46	1.0	2,190,000	8,200	42	50	–	3	2	3	–	++++	–	–	–	++
June 2	May 6	41	1.0	2,090,000	11,200	49	39	–	1	8	3	–	++++	++++	2	–	–
" 9		57	1.4	2,070,000	6,600	36	51	–	2	8	3	–	++	++	–	–	–
" 16		53	1.2	2,200,000	8,400	24	67	–	2	5	2	–	+++	++	1	–	++
" 23		49	1.1	2,110,000	7,000	26	68	–	2	3	1	–	++	–	–	–	++
" 30		35	0.9	1,850,000	6,400	26	68	–	2	1	3	–	++++	–	1	–	–
July 8		42	1.2	1,710,000	7,000	22	72	–	1	3	2	–	++++	–	1	–	–
" 14		39	1.3	1,470,000	7,800	42	44	–	1	11	3	–	++++	–	–	–	–
" 21		40	1.1	1,700,000	5,600	25	72	–	2	1	–	–	++	–	–	–	–

July 22 Monkey very weak. Chloroformed in order to obtain organs in fresh condition.

* Per 100 leucocytes.

between abundant fat cells. Giant cells were frequent. There was a great abundance of large phagocytic cells containing yellowish pigment. Many polymorphonuclear leucocytes were present throughout the tissue. No islands of normoblasts but many nucleated erythrocytes of about normal or slightly increased size were found. The general impression was of blood destruction with active regeneration of an irregular type. Local inflammation was indicated by the leucocytosis. The marrow of the left tibia was more cellular, fat cells less numerous and most of the cells lymphoid mononuclears. Comparatively few nucleated reds could be made out and there were few polymorphonuclear leucocytes (Fig. 8). The marrows of the right femur and right humerus were very cellular with practically no fat cells present and no islands of red cells. Most of the cells were lymphoid mononuclears. The findings in this monkey, accordingly, differed from those in the rabbit in that in the former the marrows did not show a replacement of the marrow and fat cells by the homogeneous granular material which was such a prominent feature in the rabbit marrows but these cellular elements were replaced by lymphoid cells. As with the rabbit, however, there occurred a very marked decrease in fat cells in marrows far removed from the site of inoculation. In both species there was evidence of marked degeneration of the blood-forming cells in the marrows *throughout* the body.

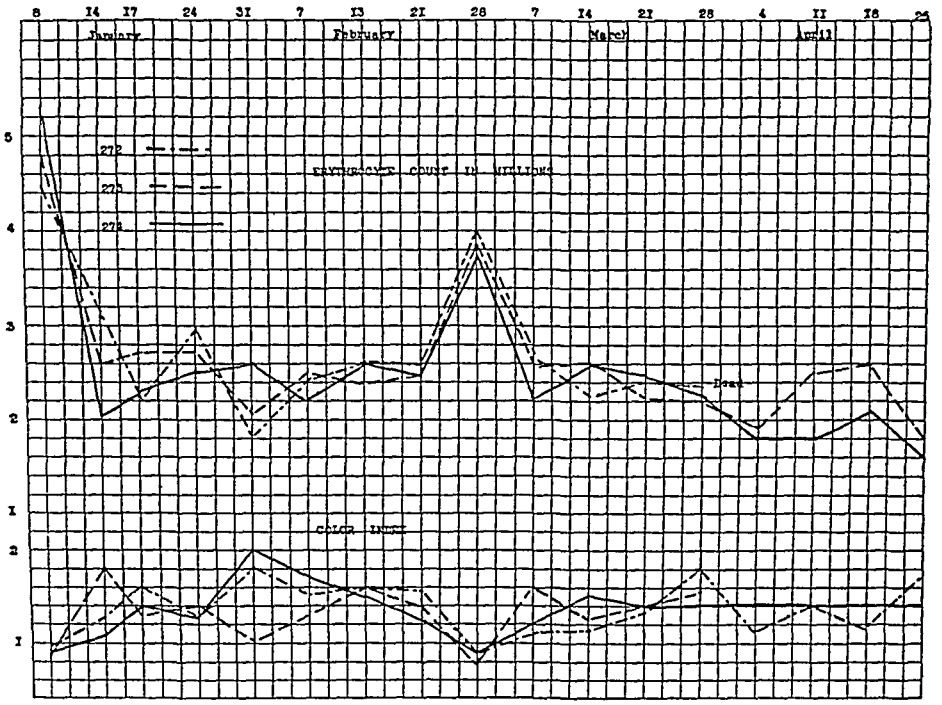
Confirmatory Tests

On January 13, 1927, three large rabbits, Nos. 272, 273 and 274, ranging in weight from 2000 to 2200 gm. were each inoculated into the left tibial marrow with 0.5 cc. of *B. welchii* toxin. This was a minimal lethal dose for a pigeon when injected into the breast muscle. One of the animals, No. 272, died seventy-four days later, repeating almost exactly the history of Rabbit 254 (death in seventy-five days). The erythrocyte count was 2,360,000 and the hemoglobin 76. Rabbit 273 was killed 102 days later in a very weak and anemic condition and without doubt would have died from its condition. The erythrocyte count was 1,830,000 and the hemoglobin 64. Rabbit 274 was in a marked to profound anemic condition for 112 days when he was killed in order to obtain the bone marrow. The erythrocyte count about that time was 1,600,000 and the hemoglobin 46. The morphological changes in the blood in all these three

rabbits were much the same as for Rabbit 254 (Table I) except that nucleated reds were not observed at any examination. Several inoculations of toxin, given intravenously in monkeys, cause after two weeks a flood of nucleated reds as described in a previous paper [2] but not when a single injection is given into the marrow. Polychromatophilia and basophilia in a moderate to a marked degree was

CHART I

Blood counts on rabbits after a single intratibial (marrow) injection of B. welchii toxin



observed at nearly every examination of the three rabbits beginning with the day following the inoculation. Transitional leucocytes and myelocytes were observed only on one or two occasions in each of these rabbits.

Chart I shows in detail the changes and fluctuations in the weekly erythrocyte counts and color indices determinations for the three rabbits. These curves have been superimposed and bring out in a striking way the almost identical reaction of the three animals to the single intramarrow inoculation of the toxin, both in degree and in point of time. The erythrocyte count dropped to between 2,000,000 and 3,000,000 the day following the inoculation and remained at that level continuously for seven weeks. There then occurred in all three animals a strong attempt at blood regeneration, as in each

the erythrocyte count rose to nearly 4,000,000 and the color index dropped to below 1.0. This lasted only a few days at the most, for on the eighth week examination the red cell count had dropped to the former level and the color indices were considerably above 1.0, as had been the case heretofore. In the case of Rabbit 254 a similar major regenerative attempt occurred in the eighth week (Table I). Minor remissions may also be noted in the third to fourth weeks and in the thirteenth to fourteenth weeks in the two surviving that long.

Rabbit 272 at death was markedly emaciated, the loss in weight being about 41 per cent. Rabbit 273, however, showed a loss of only 3 per cent. The internal organs in all were anemic but showed nothing of particular note macroscopically except a shrunken condition of the spleens. In two normal rabbits of approximately the same size, the spleens averaged 8 by 60 mm., whereas in Rabbit 272 the measurement was 5 by 15; in No. 273, 4 by 46; and in No. 274, 5 by 45. Microscopic examination showed heavy pigmentation and large microphages in the spleen of No. 272. The liver of No. 272 exhibited marked congestion with a rich deposit of fine granular yellowish pigment within the liver cells but no fatty or granular degeneration, whereas the liver of No. 273 was extremely fatty with acute universal lipoid degeneration and occlusion of capillaries from swollen liver cells. The marrows of Nos. 272 and 273 showed, in general, similar changes to those already noted for Rabbit 254, both macroscopically and microscopically. In No. 272 the changes in the right and left tibial marrow (into which the toxin was injected) were the same in type and degree, consisting of a remarkable proliferation of large cells replacing fat and causing an extensive atrophy of the blood forming cells. Among the remaining cells there were many polymorphonuclear leucocytes, a few myelocytes and many large hemoglobin-holding cells with pyknotic nuclei. There were also many pigmented microphages. The total blood-producing capacity of these marrows was greatly reduced and the type of cell formation abnormal. The right and left femurs showed similar changes but less marked. In No. 273, which lived longer than No. 272, the marrow changes in the right tibia were the same as in the left but less marked as the marrow was much more cellular and the large clear cells were less numerous. The changes in the right and left femurs were of the same character as in the left tibia but less marked.

In an attempt to determine the rapidity of the toxin action on the

bone marrows, Rabbit 500 was inoculated with 0.5 cc. *B. welchii* toxin into the left tibial marrow and then killed after eighteen hours. Before inoculation the erythrocyte count was 4,600,000 and the hemoglobin 80. Eighteen hours following inoculation the erythrocytes were 2,250,000 with marked anisocytosis and polychromophilia and slight poikilocytosis, the hemoglobin 59, color index 1.3, leucocytes 8,800. There was some hemorrhage into the left marrow. Microscopically there were the same changes in the right and left tibial marrows but curiously more marked in the right than the left. The right tibia (on the other side of the body from the inoculation) showed the tissue considerably damaged with complete atrophy of marrow cells in the center of the marrow, and marrow cells throughout much diminished in number. A good many fragmented nuclei, a great many polymorphonuclear leucocytes and numerous eosinophiles were noted; also cells showing atypical mitoses and degenerating giant cells. The fat cells were still normal and abundant. The right femur showed irregular congestion with areas devoid of cells, considerable degeneration of giant cells and many polymorphonuclear leucocytes. The left humerus exhibited the same changes as the femur with the same type of fat cells showing granular degeneration. The changes in the right tibial marrow are illustrated in Fig. 2, which may be compared with the normal marrow as shown in Fig. 1. The photographs reproduced in the plates are all from the same general region of the tibial marrow, about 1 cm. from the proximal end.

The above observations indicate that within the first twenty-four hours following an intratibial inoculation of the toxin the damage is by no means confined to the marrow into which the inoculation was made but has caused marked blood destruction, to the same degree as if the inoculation had been made intravenously, and also degenerative changes in marrows far removed from that inoculated.

Series of Rabbits Killed and Examined Twelve Days after Inoculations

A rabbit (No. 98) was inoculated into the left tibia with 0.5 cc. of toxin, killed after twelve days and the marrows examined to determine the changes occurring after that interval. Twenty-four hours after the injection, examination of the blood showed a severe condition of anemia. There was an extreme degree of anisocytosis with a strong predominance of dark microcytes, marked poikilocytosis,

many shadow erythrocytes with cell walls broken and also numerous nucleated forms. The animal was so anemic that it was only with difficulty that sufficient blood for a count was obtained from a peripheral vessel. Further details are shown in Table III. The blood destruction twenty-four hours following the intramarrow injection was far more marked than that shown after the same period by Rabbit 593, inoculated intravenously with the same dosage of toxin (See Table IV). Rabbit 98, when killed after twelve days, had decreased in weight from 1820 to 1170 gm., a loss of 650 gm. or somewhat over one-third. Autopsy did not show anything of note except a rather small spleen, 35 by 8 by 4 mm. The marrows were a bright red throughout. Microscopic examination of the right tibial marrow showed an extremely marked atrophy of marrow cells and a marked increase of fat cells, most of which had degenerated. Polymorphonuclears were extremely abundant. The same changes were observed in the other marrows examined, *viz.*, the left tibia and right and left femurs. Degenerative changes were thus well advanced at this time (Fig. 3). The blood examination of this rabbit after the twelve-day period is detailed in Table III and for comparison the findings for a rabbit (No. 593) which was given a single inoculation of the same amount, 0.5 cc., intravenously and killed after twelve days (Table IV). The erythrocyte count for this latter animal showed a drop from 4,840,000 to 3,230,000 but no change in hemoglobin. There was a moderate degree of anisocytosis, slight polychromatophilia but the blood injury was in no way comparable to that of No. 98, as is shown in Table IV. The report on the microscopic examination of the marrows of this rabbit (No. 593) is as follows:

Right Tibia: The marrow is rather cellular, with fat and cellular tissue about equal. The fat cells show mucous degeneration in moderate degree. Polymorphonuclear leucocytes are very numerous. There are a great many large mononuclear acidophile cells and many of these appear to be megaloblasts. The blood formation seems to be of megaloblastic type (Fig. 3).

Right Femur: The marrow cells are limited to a narrow ring about the periphery of the marrow. There is slight mucous degeneration of fat cells with considerable capillary congestion. The polymorphonuclear cells are rather numerous. There are an unusual number of very small pyknotic nuclei which seem to belong to nucleated reds.

TABLE III

Comparative Effect of the Intramarrow (Tibia) Inoculation of B. Welchii Toxin, Hemolysin and the Sterile Medium. Animals Killed Twelve days Later and Marrows Examined

Rabbit	Date	Material inoculated	Hemoglobin	Color index	Red blood count	White blood count	Anisocytosis	Poikilocytosis	Nucleated reds	Punctated basophiles	Polychromatophilia
	1927		*per cent*								
No. 98	May 27	None	85	1.0	4,200,000	6,400	–	–	–	–	–
Wt. 1820 gm.	June 2	0.5 cc. Toxin									
	" 3		27	1.0	1,300,000	–	++++	+++	5	–	++++
	" 13		48	1.2	1,920,000	3,800	++++	++	–	–	++
Wt. 1170 gm.	" 14	Killed									
No. 96	May 27	None	86	1.0	4,370,000	6,400	–	–	–	–	–
Wt. 1780 gm.	June 2	0.5 cc. Hemolysin									
	" 3		55	1.1	2,360,000	6,400	+	–	–	–	–
	" 13		45	0.8	2,630,000	5,000	++	–	–	–	–
Wt. 1940 gm.	" 14	Found dead									
No. 97	May 27	None	85	1.0	4,260,000	6,000	–	–	–	–	–
Wt. 1880 gm.	June 2	0.5 cc. Sterile medium									
	" 3		76	1.0	3,600,000	7,100	–	–	–	–	–
	" 13		50	1.0	2,440,000	4,600	–	–	–	–	–
Wt. 1275 gm.	" 14	Killed									

There are many large acidophile cells and some of these are eosinophile and granular. There are some large nucleated reds.

Right Humerus: About half the marrow is cellular and half fat tissue. The cellular marrow is more cellular than in the other specimens. Polymorphonuclear and eosinophile cells are very numerous. There are also a great number of small pyknotic nuclei belonging to the series of normoblasts, but few large nucleated reds are seen. The fat cells show little change.

The changes were thus much more delicate than in Rabbit 98 and not of the same character, particularly as regards degeneration of the fat cells.

Rabbit 96 of this series received into the left tibial marrow 0.5 cc. of a growth product of *B. welchii* which was very strongly hemolytic but weakly toxic, as shown by inoculation into the breast muscle of a pigeon. As noted above, our "toxin" inoculated in 0.25 to 0.5 cc. dosage would kill the bird within twenty-four hours with the development of a local massive and hemorraghic edema, whereas 1 cc. of this hemolysin caused only very slight local reaction and no toxic symptoms. This hemolysin was prepared by growing *B. welchii* in the same medium as for the toxin (3 per cent peptone, 0.5 per cent glucose, casein digest broth) except that no pigeon muscle or fresh rabbit blood was added and the tube was not sealed in the blow flame. Further details in regard to the nature and antigenic properties of these two products will be given in another publication.

This rabbit was apparently in good condition eleven days after the injection of the hemolysin into the marrow. It had increased in weight from 1780 to 1940 gm. (compare with No. 98). Ten cc. of blood was taken from the ear vein. On the next day, however, the animal was found dead. Necropsy did not disclose the cause. The degree of anemia was not nearly as great as for No. 98. The spleen measured 44 by 11 by 3 mm., a little larger than that of No. 98. The marrows differed markedly from those of No. 98 in being a much darker red throughout. Microscopic examination of the left tibial marrow showed thrombosis of the main central vein with the surrounding tissues infarcted. There was also a very marked increase of large pale proliferating fat cells and a corresponding diminution of marrow cells which were not very much altered. There were many large hemoglobin-holding cells and eosinophiles. The marrow cells were not very much altered and there was not nearly as marked

TABLE IV

Blood Changes in Rabbit Twelve Days after a Single Intravenous Inoculation of B. Welchii Toxin

Rabbit	Date	Material inoculated	Hemoglobin	Color index	Red blood count	White blood count	Anisocytosis	Poikilocytosis	Nucleated reds	Punctated basophiles	Polychromatophilia
	1928		*per cent*								
No. 593	Jan. 26		85	0.88	4,840,000	9,200	tr.	–	–	–	tr.
Wt. 2960 gm.	Mar. 1	0.5 cc. Toxin intravenously									
Wt. 3240 gm.	" 13		84	1.4	3,230,000	6,400	++	–	–	–	+

atrophy as in the case of Rabbit 98, in which the marrows were far more damaged. The right femur, the side opposite the site of inoculation, showed similar changes with moderate atrophy of marrow cells and considerable proliferation of fat cells. In fact, the proliferation of large fat cells was a very striking feature in this animal, and the findings, in general, were markedly different from those in the rabbit (No. 98) inoculated with the toxin.

Rabbit 97 of this series was inoculated into the left tibia with 0.5 cc. of the sterile culture medium. Subsequent examination disclosed that the animal was not entirely normal as there was evidence of an old lesion in the right tibial marrow. The animal lost weight markedly during the twelve days, decreasing from 1800 to 1275 gm. There was also a marked decrease in the erythrocyte count and the hemoglobin, as shown in the table, but the color index remained almost exactly 1.0 and no abnormalities were observed in the stained blood films. At necropsy the spleen measured 39 by 7 by 4 mm. Other organs appeared normal. The marrows were a bright red throughout, except the distal ends of the tibias which are normally much lighter in color. The right tibia, the site of an old lesion, showed a marked proliferation of fat cells but the changes were much less marked than in No. 98. The right femur marrow showed no change and was normal in appearance.

By way of further control two rabbits were inoculated intramarrowly with the sterile culture medium and observed for a period of about nine weeks. Rabbit 579 was inoculated into the right tibial marrow with 0.5 cc. of the sterile broth medium in which pigeon muscle had not been soaked, and No. 587 with the broth to which the muscle was added and incubated over night. The details for these animals are given in Table V. It will be noted that at the end of the period both animals had gained in weight in contrast with the very marked loss generally observed in the animals inoculated in the same way with the toxin. The erythrocyte counts and the hemoglobin fluctuated with a slight downward tendency in No. 579, but remained quite constant with No. 587. The color indices remained close to 1.0 and the blood showed no morphological or staining abnormalities throughout the experiment. Both animals appeared in fine condition at the termination of the experiment. The report on the right and left tibia and the right femur marrows of these rabbits is as follows:

TABLE V

Control Rabbits. Effect of Intramarrow (Left Tibia) Inoculation of Sterile Culture Medium Without Pigeon Muscle (No. 579) and with Pigeon Muscle (No. 587)

Rabbit	Date	Material inoculated	Hemoglobin	Color index	Red blood count	White blood count	Anisocytosis	Poikilocytosis	Nucleated reds	Punctated basophiles	Polychromatophilia
	1928		*per cent*								
No. 579	Feb. 6	None	90	0.9	4,960,000	11,000	–	–	–	–	–
Wt. 2972 gm.	" 7	0.5 Sterile culture medium									
	" 14		85	1.0	4,010,000	7,000	–	–	–	–	–
	" 28		82	1.0	4,240,000	14,600	–	–	–	–	–
	Mar. 6		84	1.0	4,320,000	5,600	–	–	–	–	–
	" 13		82	1.0	4,270,000	7,000	–	–	–	–	–
	" 27		85	1.0	4,070,000	8,600	–	–	–	–	–
Wt. 3225 gm.	Apr. 10		81	1.0	4,080,000	4,200	–	–	–	–	–
No. 587	Jan. 26	None	85	0.9	4,850,000	6,200	–	–	–	–	–
Wt. 2675 gm.	Feb. 2	0.5 cc. Sterile culture medium*									
	" 9		88	0.9	4,910,000	8,200	–	–	–	–	–
	" 16		87	1.0	4,690,000	6,000	–	–	–	–	–
	" 23		87	1.0	4,500,000	6,600	–	–	–	–	–
	Mar. 1		82	1.0	3,950,000	7,200	–	–	–	–	–
	" 8		84	0.87	4,800,000	7,200	–	–	–	–	tr.
	" 13		78	1.1	3,520,000	8,400	–	–	–	–	tr.
	" 29		82	0.9	4,620,000	9,400	–	–	–	–	–
Wt. 3075 gm.	Apr. 12		89	1.0	4,470,000	9,800	–	–	–	–	–

* Sterile pigeon muscle was added to the medium and incubated overnight.

"There is a marked reduction of all types of cells. The fat cells are normal. The femur marrows are slightly more cellular than the tibial. There is no other change in the marrow except simple atrophy. The condition is probably physiological and preëxisting." The appearance of the right tibial marrow of Rabbit 579 is shown in Fig. 7. This cellular atrophy may possibly have been induced by the medium ingredients, particularly the peptone, but the change was not sufficient to affect the blood picture and was in no way comparable to that following the toxin.

It seems evident, then, that medium ingredients were not responsible for the progressive degeneration of the hematopoietic system following the intratibial inoculation of the *B. welchii* toxin.

In the case of one monkey it was determined that the immunity following a long series of intravenous inoculations of *B. welchii* toxin included also the marrow. This monkey (J) had received twenty-eight treatments between January 14, and March 12, 1926, in dosage ranging from 1 cc. to 20 cc. After a prolonged period of anemia the blood picture returned to approximately normal. The animal was allowed to rest for a month and was then given 1 cc. of *B. welchii* toxin into the tibial marrow. Blood examinations during the following two weeks indicated the treatment had no effect whatever and it was thus evident that the immunity produced by the intravenous inoculation extended to such a susceptible tissue as the bone marrow.

Intravenous Inoculation of *B. Welchii* Toxin and Hemolysin

For purposes of comparison with the effects of the intramarrow inoculations, a description is given of blood and marrow changes following the intravenous inoculation in rabbits with the same sterile *B. welchii* growth products. Three rabbits, Nos. 112, 114 and 269, were given five injections of 1 cc. dosage at weekly intervals and finally an intraperitoneal inoculation of 10 cc. Rabbit 112 received the "hemolysin," Rabbit 114 the toxin and Rabbit 269 a mixture of equal parts of the two. The toxin was only feebly hemolytic when tested *in vitro*, 1 cc. causing at the most partial lysis of 1 cc. washed (5 per cent) sheep cells after one and a half hours at 37° C., whereas the "hemolysin" prepared by growing the *B. welchii* in the same broth medium but without pigeon muscle or blood and with the tube

TABLE VI

Blood Changes in Rabbits Inoculated Intravenously with Sterile Toxic Products of B. Welchii Growth

Rabbit	Date	Material inoculated	Hemoglobin	Color index	Red blood count	White blood count	Anisocytosis	Poikilocytosis	Nucleated reds	Punctated basophiles	Polychromatophilia
	1927		*per cent*								
No. 112	Mar. 23	Hemolysin	92	0.97	4,710,000	10,800	–	–	–	–	–
Wt. 2,670 gm.	11.05 a.m.	1 cc.									
	4.05 p.m.		66	1.0	2,670,000	5,200	+	–	–	–	–
	Mar. 24		66	1.0	3,260,000	17,000	++++	–	–	–	–
	" 26		51	0.8	3,060,000		++++	–	–	–	–
	" 30	1 cc.	61	2.3	1,390,000	5,000	++	+	–	–	+++
	Apr. 6	1 cc.	69	1.0	2,710,000	5,500	++	–	–	–	+
	" 11	1 cc.	76	1.7	2,110,000	8,400	++	–	–	–	–
	" 20	1 cc.	64	1.7	1,940,000	11,000	+++	+	–	–	+++
	" 27	10 cc. Intraperit.	60	0.75	4,060,000	12,600	++	+	–	–	+++
Wt. 2,445 gm.	May 4		65	1.0	3,020,000	7,000	++	–	–	–	+
No. 114	Mar. 23	Toxin	92	0.97	4,740,000	9,200	–	–	–	–	–
Wt. 2,640 gm.	11.25 a.m.	1 cc.									
	4.25 p.m.		70	1.3	2,670,000	7,800	++	–	–	–	–
	Mar. 24		66	1.1	2,900,000	17,200	++++	–	–	–	–
	" 26		53	1.2	2,290,000		+++	–	–	–	–
	" 30	1 cc.	72	1.8	2,090,000	4,800	++++	++	–	–	+++
	Apr. 6	1 cc.	59	1.0	2,800,000	6,200	+++	+	–	+++	++
	" 11	1 cc.	79	2.1	1,890,000	3,000	+++	–	–	–	–
	" 20	1 cc.	74	1.7	2,160,000	7,200	+++	+	–	–	–
	" 27	10 cc. Intraperit.	56	0.72	3,990,000	10,600	+++	–	–	–	++
Wt. 2,440 gm.	May 4		57	1.0	2,660,000	4,800	++++	–	–	–	++++
No. 269	Mar. 23	Toxin-hemolysin	81	0.9	4,580,000	9,800	–	–	–	–	–
Wt. 2,610 gm.	11.50 a.m.	1 cc.									
	4.50 p.m.		61	1.6	1,870,000	11,200	–	–	–	–	–
	Mar. 24		55	1.0	2,750,000	10,200	++++	–	–	–	–
	" 26		50	0.9	2,730,000		+++	–	–	–	–
	" 30	1 cc.	56	3.0	910,000	4,000	++++	–	–	–	+++

unsealed, was very potent *in vitro* causing complete lysis of 1 cc. of the washed sheep cells in dosage of 0.05 to 0.025 cc. within one hour at 37° C. One week following the first inoculation the greatest amount of blood destruction was observed in No. 269, receiving the mixture. Comparing the toxin and the hemolysin, a somewhat greater degree of blood destruction was noted in one hour following the inoculations of the hemolysin (Rabbit 112) than the toxin (Rabbit 114); as shown particularly in the hemoglobin readings, Rabbit 112 hemoglobin decreasing from 92 to 66 while that of Rabbit 114 dropped only from 92 to 85. As shown in Table VI, however, this difference was more than made up after a lapse of a week, the toxin rabbit revealing then the greater degree of blood destruction but not so much as the toxin-hemolysin animal (No. 269). These differences were confirmed in another set of rabbits. In spite of repeated intravenous inoculations of these *B. welchii* products, a marked immunity developed in all within three to four weeks. This immunity was strong enough to protect the blood against the 10 cc. intraperitoneal inoculations in the toxin and hemolysin rabbits but not in the animal treated with the toxin-hemolysin mixture.

The marrows of Rabbits 114 and 269 were a dark maroon color, whereas those of No. 112 (hemolysin rabbit) were a lighter red. Microscopically the marrows of Nos. 112 and 114 did not show any definite changes from the normal except those of No. 112 which appeared somewhat more cellular.

The above animals had developed an immunity and it was quite possible that injury done to the marrows might have undergone repair. Accordingly another rabbit (No. 582) was given three intravenous inoculations of toxin within a period of twelve days and killed ten days after the last injection and while still in a markedly anemic condition, the erythrocyte count being 2,340,000 and the hemoglobin 60 with marked anisocytosis and polychromatophilia. The marrows were dark red. Sections of the right tibial marrow showed a very cellular and considerably congested condition, but there were no changes in the fat cells. All types of marrow cells appeared in normal numbers, with eosinophiles moderately numerous. There were no definite pathological changes. A description has already been given (page 131) of the findings in a rabbit (No. 593) after a single intravenous inoculation of toxin.

Discussion

The preceding experiments show clearly that a single inoculation of *B. welchii* toxin in small dosage into one bone marrow of a rabbit or a monkey causes a toxic degeneration of the cellular elements of the marrow with a consequent anemic condition persisting until the death of the animal ten weeks or more later (about 50 per cent of our rabbits died) or as long as the animal was observed, up to seventeen weeks. This degenerative change affects particularly the fat cells which seem to increase in number at first, but later undergo mucous degeneration to such a degree that they are finally entirely replaced by a peculiar homogeneous granular material. At the same time there occurs a degeneration of the marrow cells and the erythrocyte-forming cells. In the intermediate stages unusual numbers of polymorphonuclear neutrophiles and eosinophiles accumulate in the marrow. These changes begin immediately after the inoculation of the toxin and are not restricted to the marrow into which the injection is made but are quite as prominent or more so in marrows far removed therefrom, even within twenty-four hours or less following the treatment. Sufficient marrows were examined to indicate that the whole system was probably involved. Control inoculations with the sterile medium itself showed that the injury was due to the toxic growth products of the *B. welchii* and not to the medium ingredients. Such extreme marrow changes further do not follow intravenous inoculation of the toxin regardless of the dosage used or the number of inoculations. The marrows become more cellular and congested after intravenous inoculation. Following the first inoculation, the fat cells may show mucous degeneration in moderate degree but an immunity soon develops which arrests the process and permits a return to normal even though very large doses of toxin are employed (Table VI). It is reasonable to suppose that the inoculum reaches the various marrows of the body in the same or greater strength when the same dosage is injected directly into the blood stream as when inoculated into a single marrow as it is the blood and lymph which transports it in each case. If the degenerative changes were confined to the marrow receiving the toxin or were much greater there than in the marrows far removed, the severer effect would be expected because of contact with the undiluted toxin. Such, however, is by no means the case, as our observations demonstrate.

What then causes the strikingly different effect on the marrow system when the toxin is injected intravenously and intramarrowly? We have as yet no experimental data which would serve to explain the difference but the suggestion may be made that the undiluted toxin in its destructive action on the marrow tissue with which it is brought into direct contact may give rise to secondary toxic products particularly injurious to marrow tissue, and which on absorption throughout the lymph and blood are brought in contact with all the body marrows, causing as grave an injury to those farthest removed as to that into which the toxin was directly inoculated.

It would seem probable that an experimental anemia produced in this way may prove helpful in the study of the treatment of pernicious anemia, particularly as regards the use of liver extracts and their standardization. At our suggestion the methods employed in this study are now being used at the Lederle Antitoxin Laboratories in an experimental investigation of their applicability to liver extract standardization. In a preliminary report by Beard, Clark and Moses,[14] it is clearly demonstrated that liver extract feeding to rabbits with the chronic anemia produced in this way is beneficial, resulting in a prompt and marked rise in reticulocytes, hemoglobin and red cell counts. There seems also to be a proportionality between the amount of liver extract given and the increase in number of red cells and hemoglobin content. The benefit, in fact, seems to parallel that occurring in a pernicious anemia patient on liver extract. Their findings offer hope that a method for standardization of liver extracts may thus be established. Whipple[15] has found that a secondary anemia produced in dogs by repeated bleedings is only slightly benefited by feeding liver extract, although marked improvement follows the use of whole liver. It would seem, then, that our method offers a much more delicate method for potency titration. It is also suggested that this experimental anemia may provide a method for evaluating the blood regenerating potentialities of various chemical fractions of liver and other organs.

Summary

1. It has been found that the single inoculation of 0.5 cc. of a potent sterile *B. welchii* toxin into a tibial marrow of a rabbit or a monkey gives rise to a chronic, persistent and finally often fatal anemia characterized by low hemoglobin content of the blood, low

erythrocyte count and a color index generally above 1.0. Anisocytosis and at times poikilocytosis are pronounced.

2. A single intravenous inoculation of *B. welchii* toxin of the same dosage or a series of increasing doses gives rise to an anemia of the same type as that resulting from marrow inoculation but is followed within three to four weeks by an immunity and a return of the blood to normal or nearly normal condition. Such intravenous inoculation of the toxin causes some mucous degeneration of the fat cells and an increase in the cellular elements in the marrow, particularly the leucocytes, but results in nothing like the destructive action of inoculation directly into the marrow.

3. The single inoculation of 0.5 cc. of the toxin into a tibial marrow starts a degenerative process which apparently affects the whole marrow system. Definite evidence of beginning degenerative changes were noted in the marrows on the side of the body opposite the site of inoculation within eighteen hours. After twelve days the process was well advanced as shown by the marked mucous degeneration of the fat cells, great diminution in other normal marrow and blood-forming cells and a marked increase in polymorphonuclear leucocytes. In rabbits which had died eleven or more weeks after the inoculation the marrows were found to be in an advanced stage of degeneration with fat cells replaced by a granular material and an extreme atrophy of the cellular elements. Nearly all the animals showed, at whatever stage examined, a rather more pronounced degeneration in marrows far removed than in that into which the toxin was injected. As intravenous inoculation of like or larger amounts of toxin causes no more than a transitory marrow injury, the cause of the difference in effect is not clear.

4. It is suggested that the chronic experimental anemia, produced in this way may prove a useful means for evaluating the potency of extracts of liver and other organs as blood regenerating agents.

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DESCRIPTION OF PLATES

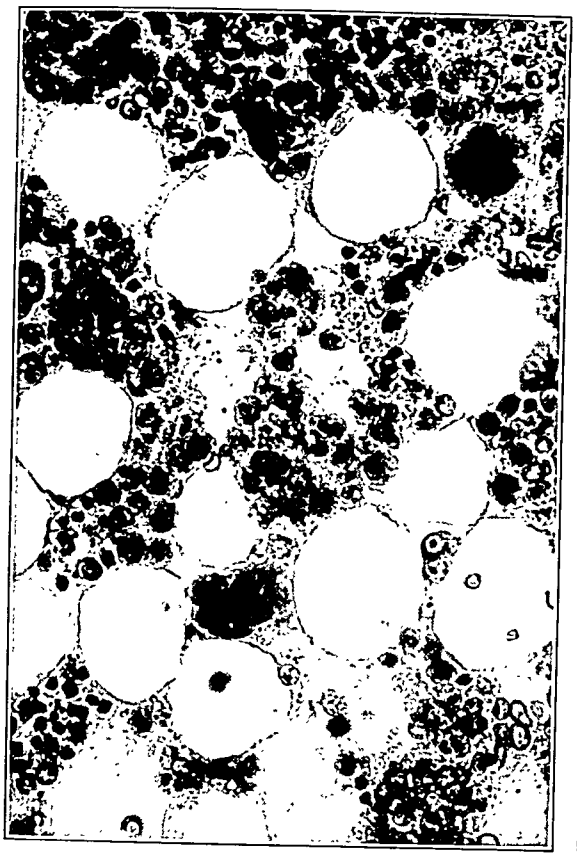
PLATE 29

FIG. 1. Normal rabbit, right tibial marrow. × 160.

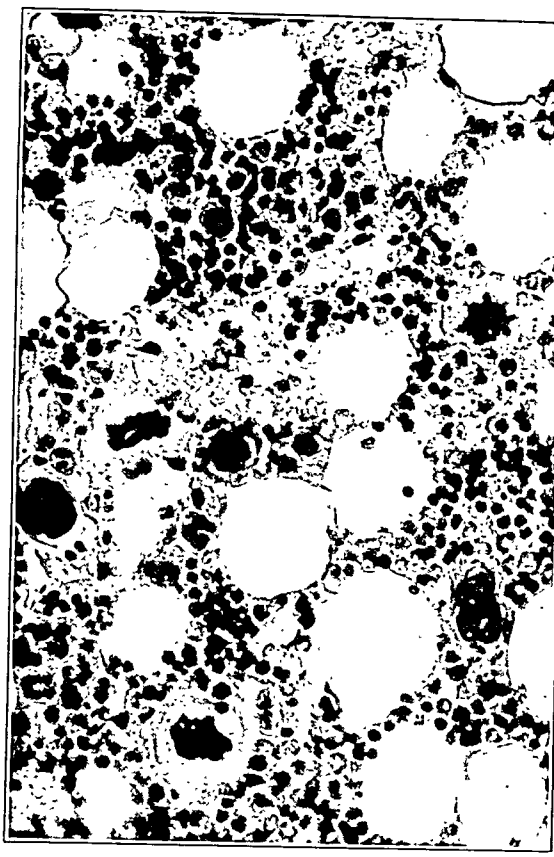
FIG. 2. Rabbit 500. Right tibial marrow. Inoculated into left tibial marrow with 0.5 cc. *B. welchii* toxin. Killed after eighteen hours. Atypical mitoses and degeneration of giant cells, many polymorphonuclear leucocytes. × 160.

FIG. 3. Rabbit 98. Right tibial marrow. Inoculated into left tibial marrow with 0.5 cc. *B. welchii* toxin. Killed after twelve days. Extremely marked atrophy and degeneration of fat cells, numerous polymorphonuclear leucocytes. × 160.

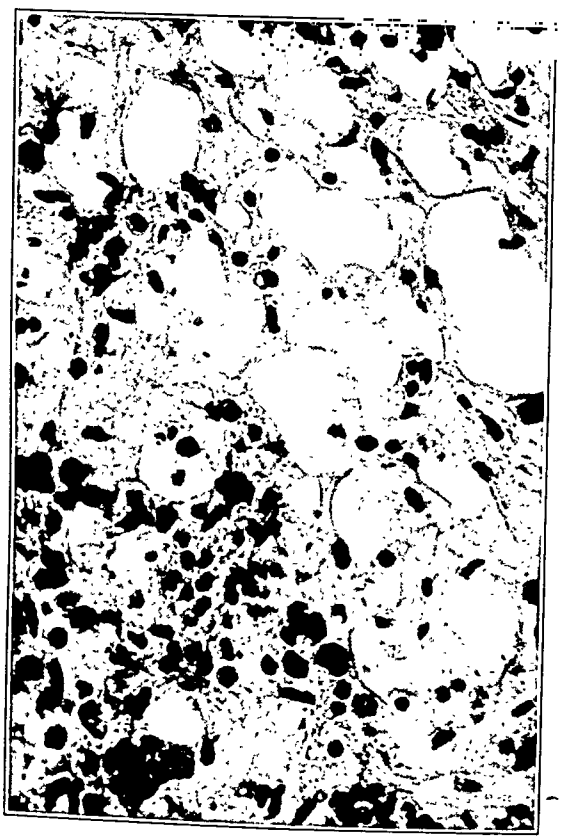
FIG. 4. Rabbit 593. Right tibial marrow. Inoculated intravenously with 0.5 cc. *B. welchii* toxin. Killed after twelve days. Marrow rather cellular. Fat cells show mucous degeneration in moderate degree. Polymorphonuclear leucocytes very numerous. × 160.



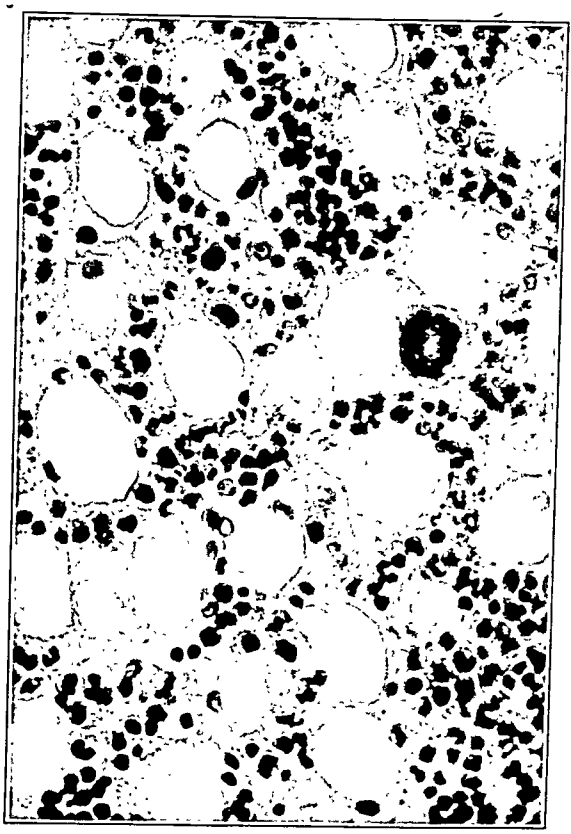
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Torrey and Kahn

Progressive Anemia Following B. Welchii Toxins

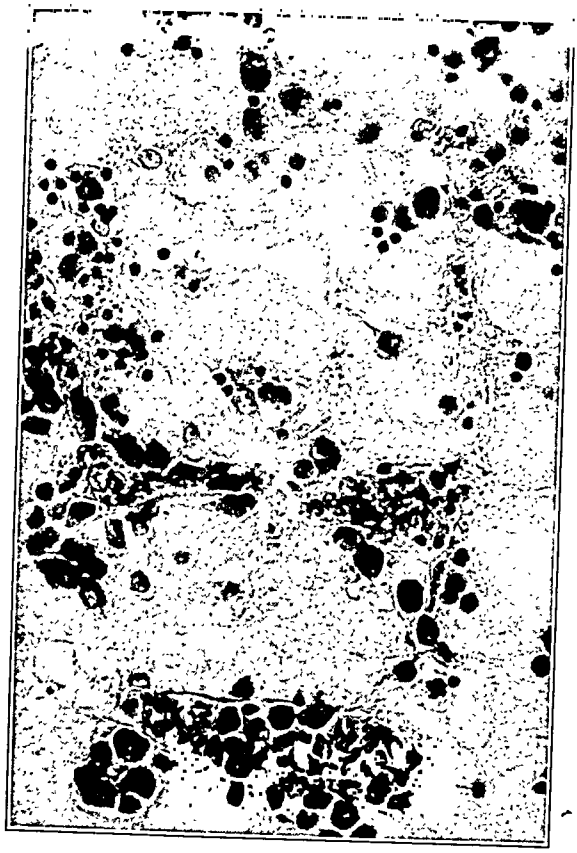
PLATE 30

FIG. 5. Rabbit 254. Left tibial marrow. Inoculated into left tibial marrow with 0.5 cc. *B. welchii* toxin. Died seventy-five days later. Replacement of fat and lymphoid cells by a homogeneous granular material. The few cellular elements are mostly myelocytes and polymorphonuclear leucocytes.

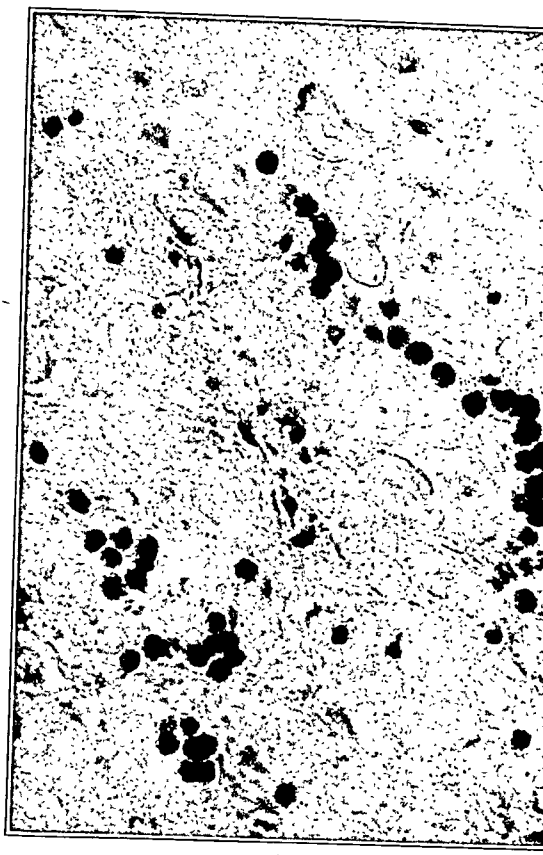
FIG. 6. Rabbit 254. Right tibial marrow. Degenerative changes even more pronounced than for left tibia. Very few cells.

FIG. 7. Rabbit 579. Control for medium. Right tibial marrow. Inoculated into right tibial marrow with 0.5 cc. sterile broth medium. Killed after sixty-four days. No change except a simple atrophy with a marked reduction of all types of cells. Fat cells are normal.

FIG. 8. Monkey Q. Left tibial marrow. Inoculated into right tibial marrow with 0.5 cc. *B. welchii* toxin. Killed after seventy-seven days. Marrow very cellular, comparatively few fat cells and very numerous lymphoid mononuclear cells.



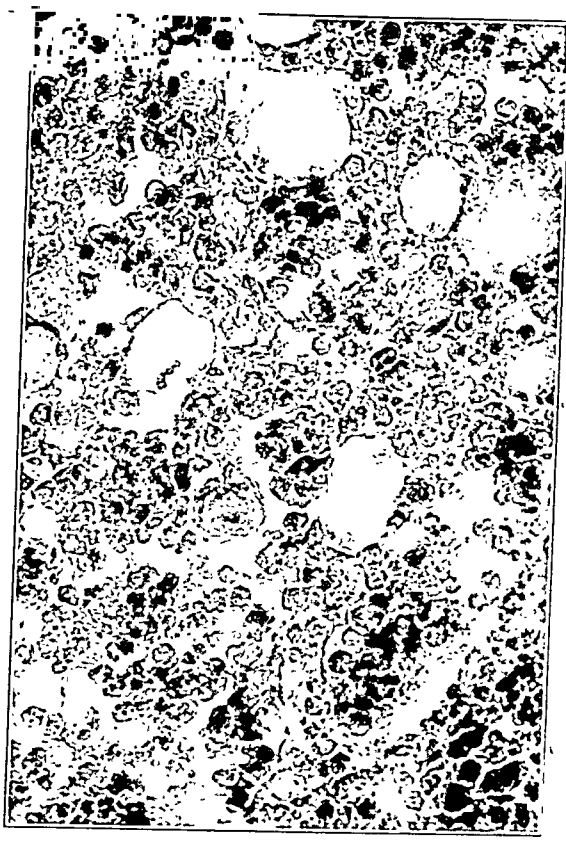
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Torrey and Kahn

Progressive Anemia Following B. Welchii Toxins

HEMANGIOBLASTOMA OF THE CEREBELLUM (LINDAU'S DISEASE)*

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Beginning his study with two cases of cystic tumor of the cerebellum similar to the one about to be described, then searching the literature for other examples, Arvid Lindau,[1] the Swedish pathologist, first proved in 1926 the hemangiomatous nature of the tumors associated with these cysts, then showed the frequent association of these cystic tumors with angiomas of the retinae (von Hippel's disease) as well as frequent hemangiomatous and cystic tumors of the pancreas, kidneys, epididymi and other parts of the body.

That the combination of these various cysts and tumors in a single patient who frequently shows hereditary predisposition forms a definite syndrome, and may properly be known by the name of its discoverer, cannot be denied. The question may be asked, however, how many of the changes included in this syndrome must be present in a patient before his ailment may be properly classified as Lindau's disease.

In my case there was no hereditary taint, no angiomatosis of the retinae, no cysts or tumors of other organs. The cyst and tumor of the cerebellum, however, were identical with those described by Lindau. Since Lindau himself states that "the dominant and characteristic thing about this pathologico-anatomical complex is evidently the occurrence of angiomata in the central nervous system" and proposes the name "angiomatosis of the central nervous system" for it, I feel justified in considering my case one of Lindau's disease.

REPORT OF CASE

Clinical History: K. R. E., a German-American, 30 years of age, was admitted to the Bellevue Hospital, New York City, in December, 1905, complaining of headache, blindness and deafness. The headache began six months before. The loss of sight and hearing was of only one months duration.

Some unsteadiness was apparently noted but no definitely localizing symptoms. The family history was negative as far as could be determined.

* Received for publication January 2, 1929.

A month later a right parietotemporal decompression was done, and a dilated right lateral ventrical demonstrated. The operation resulted in temporary improvement.

Gradually the symptoms increased and were augmented by confusion, hallucinations and excitement.

June 22, 1906, he was transferred to the Central Islip N. Y. State Hospital, apparently severely demented; without sight or hearing, disoriented, muttering and incoherent. His optic discs showed a marked degree of choking. When stood upon his feet he fell to the left. As time went on, his head became retracted permanently, he became emaciated, and finally died August 26, 1907, of pulmonary infection.

Autopsy

A complete postmortem examination was made except for the spinal cord and retinae. Aside from pneumonia, nothing unusual was found in the neck, chest or abdomen.

Brain: The brain was large with a protrusion in the right cerebral hemisphere corresponding to the decompression. The lateral ventricles were markedly dilated.

The right cerebellar hemisphere was much larger than the left, displacing the pons and medulla toward the left. The pons was swollen and grooved by the basilar artery. The medulla was much compressed and the pyramids and olives flattened. The whole right cerebellar lobe was fluctuant and the folia more flattened than on the left. A small conical process of the tumor was apparent on the base of this lobe. A section through the protruding mass showed a tumor 4 cm. in diameter. The center was cystic and filled with viscid gelatinous fluid. The solid part of the tumor was reddish brown, hemorrhagic, of loose and spongy consistency. It seemed to be entirely encapsulated and could be easily shelled out (Fig. 1). Surrounding the growth was an area of tissue destruction, probably a result of pressure of the tumor. The cyst appeared to be entirely surrounded by tumor, even where the wall was much thinned out.

Grossly, the cut surface of the tumor showed the presence of small lacunae varying from a pin-point to a pin-head in size (Fig. 1).

Microscopic Examination: Low power examination shows the growth to be characterized by the presence of many smoothly lined lacunae of varying sizes with richly cellular tissue in between (Fig. 2). With slightly higher magnification the number of spaces proves to be much greater, and definite endothelium is seen lining them. Some of these spaces are filled with red blood corpuscles while others are entirely empty. Some of the larger spaces are actually cavernous

in character and possess, outside of the endothelium, a condensation of collagen fibers.

The endothelial cells of the larger vessels are flat with elongated nuclei as seen in ordinary blood vessels. In the smaller tumor capillaries the lining cells have rounder, larger, paler nuclei, which frequently protrude into the capillary luminae and tend to obliterate them.

The cells lying between the capillaries make up a considerable part of the tumor. They show no definite cell boundaries and have fairly large, oval, pale nuclei with evenly distributed scanty chromatin. The nuclei vary moderately in size and contain among them some that are several times larger than the average (small giant cells of Lindau).

With fat stains most of the cells show coarse discrete fat globules, which Lindau thinks makes them resemble xanthoma cells (Fig. 3).

Areas of homogenous, faintly staining colloid-like material are occasionally seen, representing in Lindau's opinion, evidence of insufficient blood supply (Fig. 4).

Connective tissue stains show collagen fibers in the walls of the larger sinuses and occasional fibers between the capillaries. No glia fibrillae are demonstrable within the tumor, and no astrocytes or spongioblasts are seen in preparations made with Cajal's gold sublimate impregnation.

Discussion

The identity of the tumor in this case with the hemangiomas of the cerebellum described by Lindau is obvious when compared with his summary of the characteristics of these tumors, which is:

1. Relatively small size.

2. Cortical or subcortical position in the lateral and posterior aspect of the cerebellum.

3. Sharp demarcation of the tumor from the brain substance.

4. Microscopic findings:

(*a*) Capillary hyperplastic hemangioma.
(*b*) Cavernous spaces.
(*c*) Pseudoxanthomatous cells.
(*d*) Small giant cell.
(*e*) Plasmatic transudate.

The occurrence of this type of tumor, though obviously rare, is proving, nevertheless, to be more common than one might think. In Lindau's monograph, for example, which was published in 1926, fifteen cases are cited, including Lindau's own as well as the cases from the literature. Within a year he was able to report upon four additional cases.[2]

Among Dr. Cushing's cases eleven examples of cerebellar hemangioblastoma are present. Three of these were autopsied and showed no complications outside of the cerebellum as was true of my case. Another of Dr. Cushing's cases which had been successfully operated upon was described in detail by him and Bailey.[3] This patient had an associated hemangioma of the retina, and also gave a history to the effect that his father and his father's sister had died of cystic tumors of the cerebellum.

As to the nature of these cystic hemangiomatous tumors, Lindau identifies them with the peritheliomas of Cushing and agrees with the latter "that certain of the rare tumors, like the peritheliomas, which arise from over the fourth ventricle may originate in the structures which Wislocki and Putnam have disclosed" in the *area postrema*. This is borne out by the fact that they occur in the brain only in relation to the rhomboid space, either in the posterior aspect of this space or in the lateral and posterior parts of the cerebellum. They never occur in the superior vermis where cerebellar gliomas have their favorite seat.

Summary

The practical importance of a knowledge of these tumors is in the first place that, where they occur alone, they are completely removable surgically with a resulting cure of the condition. Secondly, in the presence of all tumors of the cerebellar hemispheres, one must, knowing the associated lesions in cases of Lindau's disease, be on the lookout for *angiomatosis retinae* and tumors of the medulla, spinal cord, pancreas, kidneys, adrenals, epididymi, etc., especially if material gained by operation proves histologically to be hemangiomatous in character. The prognosis in the complicated cases should be very guarded even if the cerebellar tumor is removed in its entirety.

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DESCRIPTION OF PLATE

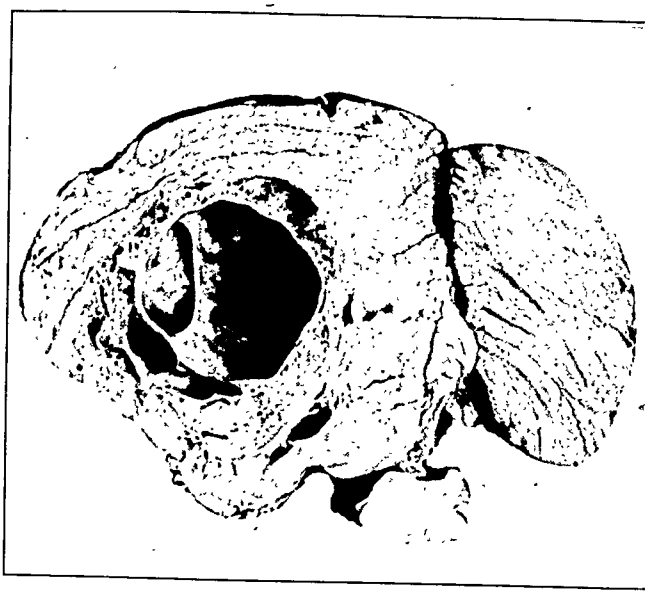
PLATE 31

FIG. 1. Actual size. Completely encapsulated cystic tumor of right lateral lobe of the cerebellum. The larger sinuses are easily discernible as small lacunae.

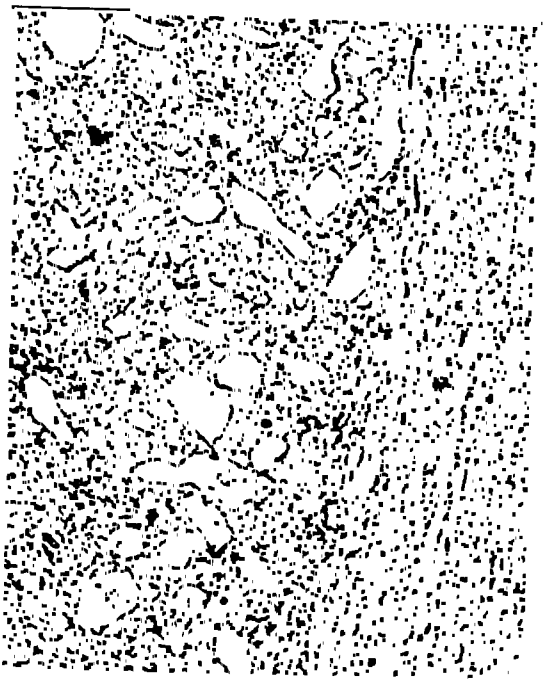
FIG. 2. The sharp demarcation of the tumor from the brain tissue may be seen, as well as the hemangiomatous character of the growth. H and E. × 80.

FIG. 3. Showing the discrete coarse fat granules in the tumor cells. Fettpenceau. × 290.

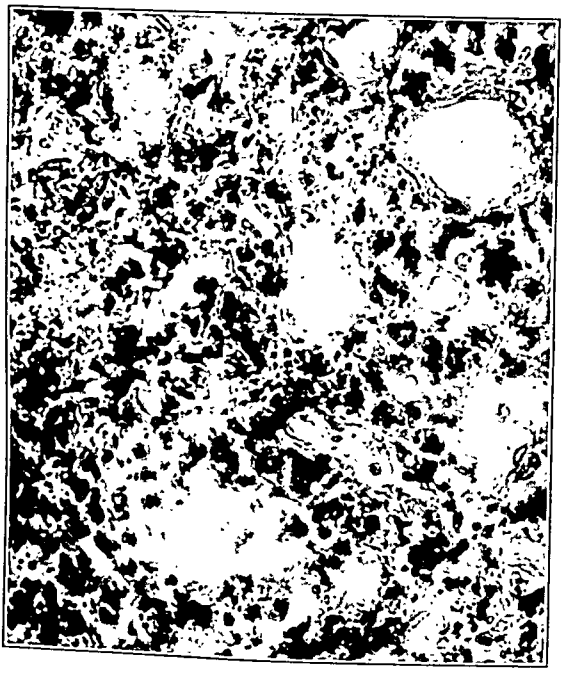
FIG. 4. An area of colloid-like coagulum seen here and there in these tumors. H and E. × 290.



1

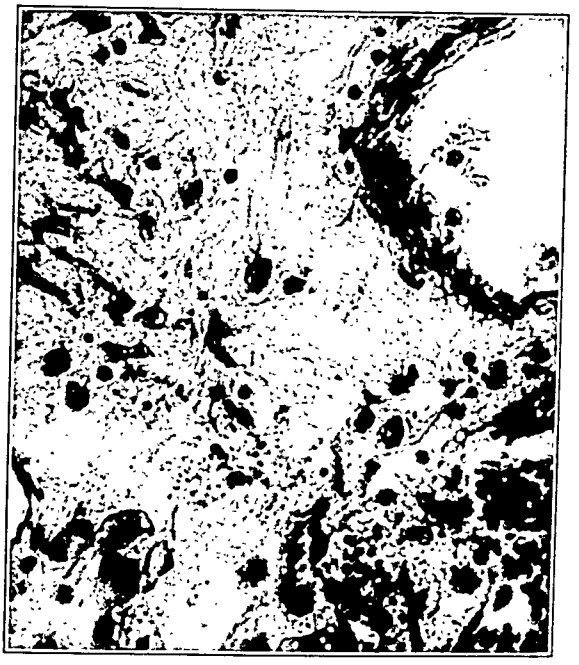


2



3

Davidoff



4

Hemangioblastoma of Cerebellum

STUDIES ON FILTERABLE VIRUSES *

III. FURTHER OBSERVATIONS ON VACCINE VIRUS

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In two previous papers,[1,2] we have described certain experiments in which vaccine virus was successfully cultivated *in vitro*. It is our intention in this paper to report further studies on some of the cultural, biological and immunological aspects of this virus. Gordon [3] has recently published his exhaustive study of vaccine virus. In this, he has covered several points that we take up but since he was working with calf or rabbit lymph, while we used culture virus, we feel it worth while to report our results.

INCREASE OF VIRUS IN CULTURE

As described in one of the foregoing papers,[2] a strain of vaccine virus (VC 10) was cultivated for a period of fifty-six days, during which time it was transferred eleven times. Titrations of the virus content were made at various times and it was found that the eleventh generation contained 51,000 times as much virus as the original inoculum. We decided to repeat this experiment using the same methods for cultivation and titration.

The culture (VC 30) was started from bits of infected testis and normal plasma. The results are given in Table I. The actual virus content at the start was not determined, but after seven days incubation, it was equivalent to fifty skin doses per culture. The rate of increase on the whole was similar to that found in the previously described culture, VC 10. The twelfth generation of VC 30 contained a very large amount of virus. One-tenth of a cubic centimeter of a 1:50,000 dilution of two cultures gave a positive take. Since a culture weighed approximately ten milligrams or one-hundredth of a gram, a gram of this culture contained 25,000,000 skin doses. After the twelfth generation the potency rapidly diminished. The thirteenth contained only one-fifth as much virus, and the fifteenth less than one-hundredth as much virus as the twelfth gen-

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eration. Thereafter even the whole dilution failed to give a take. Such a record again demonstrates clearly that multiplication of the virus takes place under such conditions of cultivation.

The sudden drop in potency of VC 30 after about three months cultivation checks quite closely with another culture (VC 6A) with

TABLE I

Growth and Titration of Vaccine virus Culture, VC 30

Generation	Date	Split	Titration		Taking skin doses per culture	Virus content
			Amount used	Result		
	Aug.					
I	4		2 cultures	0.1 cc. 1:10+ 0.1 cc. 1:100−	50	1
II	11					
III	18					
IV	25		1 culture	0.1 cc. 1:500+ 0.1 cc. 1:1,000 not done	5,000	100
	Sept.					
V	1	yes				
VI	8		2 cultures	0.1 cc. 1:5,000+ 0.1 cc. 1:10,000±	25,000	1,000
VII	15	yes				
VIII	19					
IX	26		2 cultures	0.1 cc. 1:5,000+ 0.1 cc. 1:10,000−	25,000	2,000
	Oct.					
X	3					
XI	8	yes				
XII	15		2 cultures	0.1 cc. 1:50,000+ 0.1 cc. 1:100,000 not done	250,000	40,000
XIII	22	yes	1 culture	0.1 cc. 1:5,000+ 0.1 cc. 1:10,000−	50,000	16,000
XIV	29					
	Nov.					
XV	5		1 culture	0.1 cc. 1:50− 0.1 cc. 1:10 not done	500	320

which positive skin takes were not obtained after ninety-seven days incubation. The reasons for such decreases in potency are at present obscure. They might be any one or all of these three factors: (1) diminution in virulence of the virus even although growth continues; (2) cessation of growth; (3) development of viricidal properties on the part of the tissues in the cultures. Further studies are necessary to elucidate this problem.

Certain Factors Involved in Virus Growth

(*A*) *Necessity of Living Tissue:* Having convinced ourselves that growth does take place beyond question, we then attempted to ascertain some of the factors involved.

Steinhardt and Lambert [4] concluded that living tissue was necessary for the growth of the virus. In certain of our control experiments in which the tissue was not washed or renewed and therefore died, we found that the virus did not survive. This was in accordance with the above-mentioned workers' views. In order to demonstrate this point more conclusively, cultures were set up using testis killed by freezing with a control of living testis. The cultures were set up with pieces of normal testis (one set frozen, the other untreated) and plasma to which had been added a known amount of culture virus. The control showed a strongly positive take in the first generation after incubation, whereas the culture made with killed testis was negative in both the first and second generations. Such a result confirms the view expressed above: namely, that living tissue is essential not alone for the growth but even for the survival of the virus. It also would argue against the view that the increase of virus in cultures is not due to multiplication but due to disintegration of inclusion bodies with the consequent setting free of the virus.

(*B*) *Anaerobiosis:* Plotz [5] in 1922 described the successful cultivation of vaccine virus in Smith-Noguchi tubes in which conditions closely approaching complete anaerobiosis must have been obtained. We therefore attempted to determine the effect of such conditions applied to our methods. As our culture dishes were sealed with vaseline and contained growing tissue, it seemed possible that the oxygen in the cultures might be exhausted and that the virus consequently was growing anaerobically. Therefore, the air in such a plate was analyzed for its oxygen and carbon dioxide content. It was found that oxygen was present and was but slightly lower than that of air under normal conditions; the carbon dioxide was slightly increased. Hence our cultures were not anaerobic. Next, to study this point further, we incubated cultures in an atmosphere devoid of oxygen. In no instance was there evidence of growth or even survival of the virus, for we were never able to get a positive take even with the whole dilution of the first generation. This apparent disagreement with Plotz' results made it seem advisable to attempt to repeat his

work. Using exactly his technic, we repeated his experiments with absolutely negative results. With an original inoculum many times as potent as his, we were unable to demonstrate any increase or survival of the virus. Since it seemed probable that testis would be a more favorable tissue than kidney, several series of tubes were set up using this tissue. A questionable take was obtained in one tube in the third generation but the duplicate tube was negative as were later generations. In short, all our attempts to repeat Plotz' work were unsuccessful.

Effect of Temperature

Using culture vaccine virus, we carried out some experiments planned to show the effect of different temperatures on the survival of the virus. In every instance the virus content in skin doses was known. The results are given in Table II. Twenty minutes at 55° C apparently completely destroyed the virus while shorter

Table II

Survival of Virus at Various Temperatures

Temperature	Duration	Result
55° C	5 min.	+++
	10 min.	++
	20 min.	−
37.5° C	24 hours	−
6° C	14 days	++++

In this and the succeeding tables, the +'s indicate the degree of intensity of the positive takes; a negative take is shown by the sign −.

periods showed decrease in potency. Gordon [3] found that thirty minutes at 55° C completely destroyed most samples of calf lymph although a few gave weak takes after such treatment. It is interesting to speculate whether such diminution is due to diminished virulence of all the virus or to whether an increasing proportion is killed and the takes are due to the few surviving. After twenty-four hours at 37.5° C no takes could be obtained while at 6° C the virus survived at least fourteen days. The suspensions of the virus in each instance were made in Locke's solution.

Influence of Hydrogen Ion Concentration

Culture virus suspensions of known strength were made in 50 per cent glycerine containing 5 per cent disodium phosphate, adjusted to the desired hydrogen ion concentration. The final dilutions were such that 0.1 cc. originally contained 1,000 skin doses. The tubes were kept at 6° C. The results are given in Table III. At pH 5.0, the

Table III

Survival of Virus at Various $[H]^+$ at 6° C

$[H]^+$	2 days	7 days	14 days	28 days
8.0	++++	++++	±	–
7.0	++++	++++	+++	–
6.0	++++	++++	++	–
5.0	++++	+++	–	–

virus content was diminished after seven days and no virus could be demonstrated after fourteen days. At pH 6.0 and pH 8.0, after seven days the virus was unchanged but at fourteen days was markedly weaker, especially in the pH 8.0 tube. At pH 7.0, the potency was undiminished after seven days and but slightly decreased after fourteen days. All tubes were negative after twenty-eight days. It would appear that the optimum pH for the survival of the virus in glycerine at icebox temperature is in the neighborhood of pH 7.0.

Effect of Centrifugalization

If vaccine virus is particulate, it is reasonable to expect to concentrate it by centrifuging. MacCallum and Oppenheimer [6] have described a differential method for such concentration. In our experiments a culture virus containing less than five skin doses per 0.1 cc. was centrifuged at about 2,800 revolutions per minute for one hour. The fluid at the top gave a moderately positive take when undiluted but diluted 1:10 was negative. The fluid at the bottom gave a strongly positive take and, diluted 1:10, a weak but definite take. Gordon [3] obtained similar results. In one of his experiments, the fluid before centrifuging was positive up to a 1:10,000 dilution, whereas after centrifuging, the top layer was positive only up to 1:1,000 while the botton layer gave a take at 1:50,000. Such evi-

dence would favor either the particulate nature of the virus or the association of the virus with particulate matter.

Filtration

Casagrandi [7] in 1909 was the first to filter vaccine virus successfully through a porcelain filter. Since then, both successful and unsuccessful results have been reported. Since we were working with a culture virus free from cells, we felt that conditions were favorable for filtration if such a thing was possible. Two experiments were carried out using a small Mandler filter. The tightness of the filter was controlled by adding to the virus suspension a loopful of bacteria from a twenty-hour throat culture on blood serum. Subcultures of the filtrate to plain broth were negative. The first virus suspension was such that 0.1 cc. contained 2,500 skin doses. The filtrate did not give a take. To rule out the possibility that the virus had passed through the filter but not in sufficient amounts to give a take, some of the filtrate was used to dilute normal plasma and cultures were set up with this and pieces of normal testis. The cultures were carried through three transfers and then tested with negative results. The second virus suspension was so diluted that 0.1 cc. contained five skin doses. All tests with the filtrate were negative. Gordon,[4] likewise, was unable to pass the virus through Berkefeld filters.

Viricidal Action of Hyperimmune Calf Serum

These experiments were made possible through the kindness of Dr. Benjamin White of the Massachusetts State Antitoxin and Vaccine Laboratory. A calf was hyperimmunized against vaccine virus by primary scarification followed by subcutaneous and then intravenous injections over a period of three months. Two weeks after the the last injection the calf was bled. Some of this serum was used by us in the following experiments. The normal serum employed was obtained from the same calf before immunization.

In the first set of experiments, cultures were set up in the usual manner except that instead of diluting the plasma with Locke's solution, it was diluted with two parts of either 50 per cent normal or 50 per cent immune calf serum. After seven days incubation each culture was ground with sand in the usual way and various dilutions were made and tested. The results are given in Table IV. The cul-

ture containing normal serum showed definite takes as high as 1:100 and questionable takes at 1:1,000 and 1:5,000 whereas that to which immune serum was added was negative at 1:10.

In order to titrate the viricidal action of the immune serum *in vitro*, varying dilutions of culture virus were incubated for one hour

TABLE IV

Viricidal Action of Hyperimmune Calf Serum in Cultures

Culture with Normal Serum		Culture with Immune Serum	
Dilutions	Results	Dilutions	Results
1:10	++++	1:10	–
1:100	++	1:100	–
1:1,000	±	1:1,000	–
1:5,000	±	1:5,000	–

TABLE V

Viricidal Action of Hyperimmune Calf Serum in Vitro
(Varying dilutions of virus + an equal amount of undiluted calf serum)

Virus Dilutions	Results with Normal Serum	Results with Immune Serum
1:1,000	–	–
1:100	+++	–
1:10	++	–

at 37.5° C with equal amounts of undiluted immune and normal serum. The various tubes were then tested as usual by intradermal injections. The results are shown in Table V. The virus was of such potency that 0.1 cc. of a 1:10 dilution contained 5,000 skin doses. The normal serum neutralized the 1:1,000 dilution but did not affect the 1:100 and 1:10 dilutions. The immune serum neutralized through the 1:10 dilution. Since 0.1 cc. of 1:1,000 virus contained $\frac{5000}{100}$ or 50 skin doses and since 0.1 cc. of normal serum neutralized this amount, then 1 cc. of normal serum could have neutralized 500 skin doses. Applying the same method of calculation, 1 cc. of the immune could have neutralized at least 50,000 skin doses.

Since the limit of viricidal or neutralizing power of the immune serum was not determined in the last experiment, another was set up using fixed amounts of virus, 0.1 cc. containing 500 skin doses, and

equal amounts of increasing dilutions of normal and immune serum. The tubes were incubated and tested as before (see Table VI). The normal serum failed to neutralize even when undiluted whereas the immune serum neutralized definitely at a 1:1,000 dilution. On the

TABLE VI

Viricidal Action of Hyperimmune Calf Serum in Vitro
(Varying dilutions of serum + an equal amount of virus, diluted 1: 10)

Serum Dilution	Results with Normal Serum	Results with Immune Serum
Undiluted	++	−
1: 10	++++	−
1: 100	+++	−
1: 1,000	++++	−
1: 10,000	++++	±

basis of this experiment, 1 cc. of normal serum could have neutralized less than 5,000 skin doses while the immune serum was capable of neutralizing as many as 5,000,000 skin doses. In the one experiment tried by Gordon, 1 cc. of immune serum neutralized 25,000 skin doses.

SUMMARY

1. Further experiments are described demonstrating the multiplication of vaccine virus in tissue cultures.

2. Vaccine virus could not be cultivated using killed tissues or under anaerobic conditions.

3. The virus when suspended in Locke's solution, was destroyed if heated to 55° C for twenty minutes or to 37.5° C for twenty-four hours.

4. The optimum hydrogen ion concentration for survival of the virus in glycerine was found to be pH 7.0.

5. After centrifugalization, the bottom layer contained more virus than the top layer.

6. The virus could not be filtered through a Mandler filter.

7. Experiments on the viricidal action of hyperimmune calf serum are described.

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NEUROEPITHELIOMA OF THE CEREBELLUM *

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The authors, in reviewing the tumors of the central nervous system in the pathological collection of the Henry Ford Hospital, studied one tumor which is so rare in incidence and point of origin as to merit a place in medical literature.

According to Bailey and Cushing,[1] neuroepitheliomas are tumors composed of primitive spongioblasts characteristically arranged in rosettes about central canals, the canals lined by a cuticular membrane bearing blepharoplasten and presenting cilia upon its inner surface, and the outer extremities of the cells fading away as tails into the surrounding tissue. These authors state that the tumor commonly arises in the spinal cord and the retina. Flexner,[2] Wintersteiner[3] and others have described the retinal type, while Schlapp,[4] Rosenthal,[5] Thielen and Zinn,[6] and others have described the spinal cord type.

Bailey and Cushing in their series of 254 classified intracranial tumors had no examples of neuroepithelioma. However, in a subsequent article, Bailey[7] reported a case of neuroepithelioma of the left lower frontal lobe. The histological picture in this case corresponds very closely to the case reported here, as is shown by the photomicrographs and by sections of the tumor which we have been permitted to examine.

Just as we were about to submit this article for publication another case of neuroepithelioma was drawn to our attention, a very recent case of tumor involving the fourth ventricle. This case was operated upon by Dr. Harvey Cushing and as yet has not been fully studied. However, the sections which we have been fortunate enough to examine seemed typical of this pathological entity. It is also very interesting to note that the inner ends of the cells forming the rosettes bore definite cilia. This is undoubtedly due to the fact that the sections examined were from a specimen removed at operation and fixed immediately after its removal. Until this case is more

* Received for publication January 4, 1929.

completely studied, it cannot certainly be classed as a neuroepithelioma of the cerebellum.

In 1926 Naeslund[8] apparently described the first recognized case of neuroepithelioma of the cerebellum. This tumor occurred in a patient 18 years of age. It was found lying in the fourth ventricle and arose from the lateral margin of the anterior medullary velum. It had invaded and destroyed the ventral surface of the cerebellum. The photographs which accompanied the article and the description of the microscopic appearance are typical of neuroepithelioma. The columnar cells about the canals showed definite blepharoplasten in the inner ends with indistinct tails streaming into the surrounding tissue. No cilia were found.

From a perusal of the vast literature on the subject of brain tumors, two other observations were found of probable examples of true neuroepitheliomas: (1) a tumor arising near the aqueduct of Sylvius described by Roussy, Lhermitte, and Cornil,[9] and (2) a tumor of the left cerebral hemisphere described by Ribbert.[10] These two descriptions give meager detail of the finer cellular structure, but the drawings of the tumor seem characteristic of neuroepitheliomas. Possibly the tumor of the left hemisphere described by Uyematsu[11] might be a sixth example but it is difficult to judge from his brief description.

The above cases were the only examples of neuroepithelioma of the brain which a careful search of the literature revealed. "Neuroepitheliomas," it is true, are described in abundance, but a perusal of the descriptions convinces one that the term is used loosely to include ependymomas and ependymoblastomas, *i.e.*, any tumor arising from ependymal tissue and having any resemblance to epithelium. Under the title of neuroepithelioma gliomatosum, Roman[12] has described a tumor arising from the third ventricle very evidently composed of ependymal spongioblasts, and it would therefore be an example of ependymoblastoma, according to the classification of Bailey and Cushing. The tumor of the fourth ventricle described by E. Silberberg,[13] is doubtless another ependymoblastoma. This tumor showed pseudorosette formation, the lumina being blood sinuses instead of true central canals. The latter author refers to a tumor of the fourth ventricle described by M. Silberberg,[14] but there is no report of the histological detail. Cash[15] described a tumor of the lower medulla and upper cervical cord under the same title

which is unquestionably an ependymoblastoma, as the tails of the cells are well stained and arranged about blood spaces. Gordinier and Carey [16] described as a neuroepithelioma a tumor of the choroid plexus of the fourth ventricle which invaded the vermis. In the photographs it is seen that the rosettes have blood spaces for lumina. This is doubtless another ependymoblastoma.

Hirsch [17] under the title of neuroepithelioma, described a tumor of the fourth ventricle. There is no detailed histological report given, but according to the illustrations the tumor, although lying in the fourth ventricle, does not arise from the medulla or cerebellum, but extends up the dilated central canal from the cervical cord. We are unable to determine from the information given what type of tumor this is. Rosenthal [5] mentioned a gliomatous tumor of the fourth ventricle which was shown him by Glücksmann.[18] He describes this as containing passages lined with cylindrical epithelium, but furnishes no other detail. He also mentions a tumor of Ströbe's [19] which was found in the cerebral hemisphere and contained spaces lined with high cuboidal and cylindrical epithelium. Due to lack of detail in these cases it is impossible to classify them.

A tumor of the fourth ventricle arising from the posterior medullary velum is described by Muthmann and Sauerbeck.[20] This tumor is composed of columnar cells which occasionally line cavities and which possess well stained tails. These tails are gathered together in more or less thick bundles which radiate toward blood spaces. The authors are uncertain as to the presence of cilia on the inner surface of these cells. No mention is made of blepharoplasten. Bailey [21] has included this tumor in his description of ependymal tumors. This tumor bears a close resemblance to the one to be described here in that there seem to be true canals and the lining cells may have cilia. However, the authors are uncertain as to the presence of cilia, and canals are few. The tails stain unusually well, which is characteristic of ependymal spongioblasts, and we therefore would consider this tumor an ependymoblastoma.

The ependymomas have been described by Bailey.[21] Mallory [22] reported a case of ependymal tumor which was doubtless an ependymoma. Bassoe's [23] tumor of the fourth ventricle was said to resemble the tumor described by Muthmann and Sauerbeck, but according to the author's statement was less differentiated. From the description it would seem to be an ependymoma.

As to the remaining tumors which we investigated in the literature, it may be safely said that there is no example of neuroepithelioma fulfilling the criteria set down by Bailey and Cushing. This includes Greenfield's [24] series of forty tumors, Bassoe's [25] series of third and fourth ventricle tumors, and Tooth's [26] series of five hundred cases.

Neuroepithelioma of the brain is therefore very rare, four or possibly five having been described in contrast to numerous ependymomas and ependymoblastomas.

Report of Case

Clinical History: J. E. S., Case No. 57347, a boy of 5 years and 4 months, was admitted to the Pediatric Service of the Henry Ford Hospital on March 18, 1925, with the complaints of "stomach spells, dizziness, and pain in the back of the neck."

Family History: Negative.

Past History: Birth normal, spontaneous. Patient was said to have been cyanotic for two days after birth. Feeding normal. For the past two weeks the patient has had hiccoughs after eating, ending in vomiting. Development normal. Diseases, pertussis, varicella, rubella, possibly scarlet fever. Habits good. Weight 38 pounds, a loss of 4 pounds in the past two weeks.

Present Illness: Previous to the last three months the patient was quite well, playful, but whined more or less, the reason for which was unknown. He also cried easily.

At Christmas, 1924, he experienced a "stomach upset," began vomiting, especially in the morning after breakfast, and in the evening after supper, sometimes during the night. He always complained of nausea before the vomiting occurred. He has been constipated during this time, and has complained of abdominal pain on using enemas. Pain in the back of the neck had been sharp, and the patient held his head to one side (which side not stated). Recently he has had pain in both arms. During the last few days the patient has complained of double vision, and has kept the right eyelid closed. He staggers as if dizzy; seems tired all the time. He has lost four pounds in weight in the last two weeks.

Physical Examination, March 18, 1925: A well developed and well nourished boy. Weight 38 lbs., estimated normal weight 40 lbs. Temperature, 98, pulse 116, respiration, 24, blood pressure, systolic 75, diastolic 55.

General Examination: Coated tongue. Large cervical and axillary glands. Chest and abdomen negative.

Neurological Examination: Eyeslits normal; nystagmoid movements on fixation either to right or left, slight strabismus; pupillary reactions normal; bilateral papilloedema. All tendon reflexes normal; abdominals present. Gait, some staggering toward the left.

March 19, 1925: On this date there were noted: Macewan's sign negative; weakness of the left external rectus muscle, and diplopia; pupils, right slightly larger than the left, regular, reaction to light and on accommodation normal.

Lateral nystagmus, on fixation either to right or left, the quick component in the direction of fixation. Bilateral choked discs, two diopters of elevation. Tongue protruded to the right. Hearing impaired on the left, the patient hearing test-sounds at a distance of two feet on the left as compared with six feet on the right. Vestibular tests were said to indicate faulty conduction in the vestibular pathways bilaterally. Romberg positive, the patient falling to the left. Gait, a tendency to fall to the left was noted. Muscular twitchings of the arms and legs were observed during the night.

March 22, 1925: On this date a definite *tache cerebrale* was noted.

Laboratory Findings: Wassermann negative. Intracutaneous tuberculin tests negative to 1/20 and 1/10 mg. Blood and urine negative.

X-rays of the skull showed slight widening of the coronal suture.

Diagnosis: On admission a tentative diagnosis of encephalitis was made, with brain tumor and meningitis as other possibilities.

The final preoperative diagnosis lay between (1) tumor of the left cerebellopontine angle and (2) tumor of the left caudal portion of the pons. The patient was transferred to the Surgical Service for ventriculograms. Ventricular puncture was performed on March 23, 1925. On entering the right lateral ventricle the cerebrospinal fluid spurted out, evidently under increased pressure, but this was not measured. X-rays taken after injection of air into the lateral ventricle showed both lateral ventricles and the third ventricle to be enlarged.

Ventricular Fluid: Pandy negative, cells 1, Kolmer negative, Lange and mastic curves negative throughout.

The patient returned from the operating room in good condition and remained so until about 5.30 A.M. on March 25, 1925, when his condition suddenly became very poor, and he died at 6.00 A.M. of the same day.

Permission was obtained for postmortem examination of the brain only.

Necropsy (No. 319) was performed at 2.30 P.M. on March 25, 1925, by Dr. C. Z. Garber. The verbatim report follows.

Necropsy Protocol

External Appearance: "The body is that of a well nourished, well developed little white boy. Embalming has been done and permission is for examination of the brain only. In the right parietal region the hair is shaved, and there is a 3 cm. long V-shaped recent surgical incision wound. It is located about 3 cm. from the midline and 7 cm. from the external occipital protuberance. Below the wound there is a small round hole in the skull.

Brain: The meninges are not thickened. The dura below the trephination wound shows a 2 mm. hole, the borders of which are slightly roughened by small blood clots. Venous sinuses are open. The brain weighs 1330 gm. Convolutions are slightly flattened, and the sulci are rather shallow. Occupying the inferior surface of

the left cerebellar lobe and extending over the pons and medulla is a whitish gray lobulated tumor mass, a little firmer in consistency than the brain itself. The pons is pushed to the right and is very much compressed on the left side by an overgrowth of tumor. The edges of the tumor are free so that the mass can be shelled out to some extent. An attempt is not made, however, to shell out the whole mass. Lobules of the tumor extend posteriorly over the vermis and down over the posterior surface of the medulla and cord. Some of the lobules actually project into the foramen magnum. The tumor measures 6 cm. laterally, 7.5 cm. anteroposteriorly. It appears to be about 2.5 cm. thick. Several brownish red areas are visible on the tumor surface, and near the right edge there is a purplish soft area. The left trigeminal nerve is very much elongated and passes around the tumor. The left abducens nerve is a little longer than the right. The left facial and acoustic nerves emerge from the tumor mass, and are entirely surrounded by it. The edge of the tumor just touches the left glossopharyngeal, vagus, and hypoglossal nerves. The basilar and left vertebral arteries are pushed to the right by the tumor, and the left vertebral seems to have been somewhat compressed. Lobules of the tumor press on the orifices of the foramen of Magendie and the left foramen of Luschka. The right foramen of Luschka seems to be partially occluded, due to transmitted pressure of the medulla against the right cerebellar hemisphere. In the right parietal region beneath the trephination wound, there is a small puncture wound in the cortex. On section, the third ventricle and the lateral ventricles are somewhat dilated. At the level of the aqueduct there is a small puncture wound in the roof of the fourth ventricle near its medial border. The pons is found to be very much compressed and pushed to the right. The fourth ventricle is distorted and in one place is not much more than a narrow slit. At this level the tumor is not actually attached to the brain. It has a fine spongy grayish white appearance, and toward the center of the mass it shows a coarse, spongy, reddish yellow area dotted with flecks of old hemorrhage. At the level of the dentate nuclei and the inferior olivary nuclei the tumor is firmly attached to and replaces the inferior part of the left cerebellar hemisphere. Its progress upward is limited by the smooth edge of the white matter, but in places it merges imperceptibly with the gray matter. There are several cysts filled with translucent gelatinous, greenish gray

material, the largest being about 1 cm. in diameter. The best preserved part of the tumor has a slightly spongy, opaque, grayish white appearance. Small hemorrhages and bright yellow flecks present a striking contrast (Fig. 1).

Provisional Anatomical Diagnosis: Glioma of cerebellum. Pressure occlusion of the fourth ventricle. Slight hydrocephalus."

A section of the tumor was stained with hematoxylin and eosin, and the histological diagnosis of glioma was made.

Microscopic Examination

Technique: Material was refixed in formalin nine hours postmortem and embedded in paraffin. Sections were stained by hematoxylin and eosin, phosphotungstic acid hematoxylin, neutral ethyl violet-orange G, Van Gieson's acid picro-fuchsin, del Rio-Hortega's first and fourth variants and Weigert's iron hematoxylin. Sections were also impregnated according to Cajal's method for non-medullated nerve fibers but carried to such a degree that the method lost its specificity. The results although serviceable were far from perfect, due to previous embalming.

Microscopic Examination: The characteristic and predominating cells in this tumor are columnar, arranged for the most part about small canals or cavities forming true rosettes (Figs. 4 and 5). The major portion of the cytoplasm, *i.e.*, the columnar portion, lies between the nucleus and the canal, while the peripheral end of the cell, that portion just beyond the nucleus, is indefinite, and fades away into the surrounding tissue. One can make out vague and indistinct fibrous processes, which arise from the cell at this point and stream out into the "interstitial" tissue. These processes are not stained by the usual procedures, although in a very few instances in the sections prepared by del Rio-Hortega's fourth variant a very few definite processes can be detected.

By using the silver pyridine technique described by Cajal for impregnating non-medullated nerve fibers, but carrying the method to a point where it lost its specificity, the tails of these cells were beautifully impregnated in one only of a series of sections (Fig. 6). This procedure was first used by Bailey in his attempt to demonstrate the cells in the case he reported. In applying the method to this case we found that the body of the cell rapidly tapered off into

a narrow filament which frequently terminated on a blood vessel and in other cases ended in a bulbous enlargement.

In all stains, but particularly in those sections stained by Mallory's phosphotungstic acid hematoxylin, the irregularity of the outer ends of the cells, and the complete absence of any external limiting membrane is emphasized (Fig. 5). The cytoplasm of these cells consists of a finely granular eosinophilic material. At the very innermost border of the cell lies a thin cuticular membrane bearing numerous darkly stained minute rod-like and spherical bodies. These are especially well seen in the phosphotungstic acid hematoxylin and neutral ethyl violet-orange G preparations. They are undoubtedly blepharoplasten (Fig. 3). (Compare with the illustration in Bailey's Atlas.)[27] On this same surface, lining the canals, can be seen in a few instances minute processes which seem to be cilia. They are indistinct, however, and may be degenerative products or other débris.

The nuclei are small and of uniform size, about 10 microns in diameter. For the most part they are spherical with a moderate amount of finely granular chromatin scattered throughout the nucleus, slightly more at the periphery than elsewhere. In a few instances one large darkly staining granule is present, but most of the nuclei have no nucleoli. A few mitotic figures are seen, but they are far from numerous.

The rosettes described above, although found throughout the tumor, are most numerous at the periphery where they predominate almost to the exclusion of all other forms of cellular organization. Toward the interior and base of the tumor numerous variations are seen. There are many cells grouped in rosette formation without a central lumen, the cells uniting with those of the opposite side. On the other hand, there are many instances of large cavities lined by these same columnar cells. The walls of some of these large cavities are thrown into many folds (Fig. 2). Occasionally a large cavity filled with red blood cells is seen. This is evidently the result of hemorrhage into the cavity, for the wall of the cavity is composed of columnar epithelium.

In the space between the rosettes and the cavities are many cells identical with the columnar cells but in which the cytoplasm has become indistinct in outline. These cells are massed together with no attempt at organization (Fig. 7).

The surface of the tumor in many places is covered by a single layer of cuboidal cells indistinguishable from ependymal cells.

Some sections of the tumor include neighboring areas of apparently normal cerebellar tissue. At the point of junction of the neoplastic tissue with the cerebellum the comparatively lightly stained nuclei of the tumor intermingle with the somewhat smaller and much darker nuclei of the granular layer of the cerebellum. This intermingling of the two tissues is such that there is no definite line of separation between them (Fig. 7). In and near these areas the tumor cells are in unorganized masses. In a few places along this line of junction marked proliferation of the capillary endothelium has occurred.

The tumor is quite vascular, containing many large and thin-walled vessels, well visualized with Van Gieson's acid picro-fuchsin stain.

The areas about the blood vessels for a short distance are entirely free of cells, and are filled with fibrillary material (Fig. 6). This is especially evident in the section impregnated by Cajal's method and less so in those by Hortega's fourth variant. In the latter the cells arranged about the smaller vessels present definite, though poorly impregnated, fine fibrous processes which radiate toward the vessel walls. This is typical of primitive spongioblasts, according to Penfield.[28]

The sections stained by the phosphotungstic acid hematoxylin method, and those by Hortega's first variant even more clearly, show areas of marked formation of glia fibers. These are formed into an intricate network which is particularly dense at some points near the periphery of the tumor. In the less dense areas of this tissue a few bipolar cells can be found in the silver-stained sections.

Throughout the tumor, but particularly in that portion farthest from its base, are many areas of degeneration. These are definitely necrotic and many show hemorrhagic infiltration. The center of the degenerated masses, some of which are large, even 1 to 2 cm. in diameter, are acellular, and at the periphery a few poorly stained nuclei are seen (Fig. 8). These degenerated areas can also be clearly seen in the gross specimen (Fig. 1).

In one section the relation of the tumor to the ependymal lining of the fourth ventricle and to the medulla are well shown. The orderly arrangement of the cuboidal ependymal cells rather sud-

denly gives way to the tumor mass. The growth for the most part is outside the ventricle. The medulla is compressed but intact.

Schlapp states that there is a central gliosis in almost all cases of neuroepithelioma of the cord. Sections from the upper cervical cord and from the fourth ventricle in this case showed entirely normal ependyma with no evidence of cellular proliferation. The glia fibers surrounding the central canal are slightly increased in number and the walls of the central canal show some slight tendency to infolding. Sections of lower portions of the cord were not obtained.

Discussion

The primitive spongioblast has been described by Bailey and Cushing and by Penfield as a direct development of the medullary epithelium of the neural tube. These spongioblasts are cells with oval nuclei, whose inner ends form the ventricular surface of the developing nervous system, while the outer extremities continue off as long tails to the external limiting membrane of the neural tube, and also, as Penfield has pointed out, to neighboring blood vessels. His [29] and Castro [30] have shown that these cells present cilia on their inner surfaces and blepharoplasten at the base of the cilia. That these cells are the cells forming the major part of this tumor there can be no doubt.

Primitive spongioblasts have been shown to be the forerunner of bipolar spongioblasts by Castro, and this fact readily lends itself to the explanation of such bipolar cells in this tumor.

The roof of the fourth ventricle has long been recognized as the site of origin of a large proportion of cerebellar tumors. Bailey and Cushing have pointed out that the indifferent cells (medulloblasts) may occasionally be found near the ependymal lining of the ventricles, and cerebellar tumors composed of such cells and arising from the roof of the fourth ventricle are very common. Twenty-five of their twenty-nine cases of medulloblastomas had this origin. Pfleger [31] has also pointed this out as a common location for heterotopic groups of cells.

It is well known that in the adult nervous system the roof and floor plate are points of arrest in the development of ependymal cells. Streeter [32] states: "In the region of the anterior median fissure of the cord . . . the neuroglia maintains its primitive ependymal

type of simple radial fibers extending from the lumen to the surface of the cord." He also points out that such primitive spongioblasts can be found in the posterior median septum of the cord. So in view of the prevalence of embryonic rests of this type of cell in the cord it is to be expected that neuroepitheliomas of the cord would be common.

Referring to the medulla and cerebellum, Streeter says: "The fourth ventricle is completely covered by the expanded roof plate consisting of a thinned-out layer of ependymal cells which is attached laterally to the border of the alar plate, the transitional line forming the rhombic lip." And continuing: "At the rhombic lip are found cells which retain their primitive embryonic appearance into adult life," and "the ependymal cells at the rhombic lip differ from the rest of the ependyma in that they continue to show active proliferation late into embryonic life. The same feature is shown throughout the whole rhombic lip but is more marked in the portion belonging to the cerebellum. . . . It is apparent that the cortex (cerebellar) formation begins at the rhombic lip."

Thus it would seem that this area would be one of the most probable for the development of neuroepitheliomas, and it seems most likely that the tumor described, originating from the lateral wall of the fourth ventricle and invading the cerebellum, arose from the rhombic lip, the resting place of primitive spongioblasts in adult tissues, and the point of origin of the cerebellum.

Considering the prevalence in this region of tumors composed of other types of embryonic cells, medulloblastomas, ependymoblastomas, and ependymomas and the existence of an area of primitive spongioblasts at this point, it is to be wondered at that neuroepitheliomas of the cerebellum, such as the present case, are not more common.

Summary

A case of intracranial tumor in a white boy, aged five years and four months, duration of three months from the first definite symptoms to sudden death following ventricular puncture, is presented. The tumor is situated in the left inferior cerebellar lobe, displacing the fourth ventricle, and overhanging the medulla and cord. It is 6 by 7.5 by 2.5 cm. in dimensions, lobulated, vascular, cystic, and with hemorrhagic infiltration in the degenerated areas. Histologi-

cally the tumor is a true neuroepithelioma, according to the criteria set down by Bailey and Cushing for this type of tumor. As such it belongs with the brain tumors described by Naeslund, Ribbert, Roussy, Lhermitte, and Cornil, and by Bailey. It is the second neuroepithelioma situated in the cerebellum to be described.

Conclusions

1. Neuroepitheliomas are composed of primitive spongioblasts, and, as such, arise from the parent cells of ependymal tissue.

2. Though more commonly arising from the ependyma of the spinal cord and from the retina, they may arise from ependyma elsewhere.

3. A review of the literature shows that the case here reported is the fifth authentic case of neuroepithelioma of the brain, and the second involving the cerebellum.

Note: We wish to thank Dr. Frank W. Hartman for having placed the pathological material and the facilities of his laboratories at our disposal, for the photomicrographs which he made, and for his kindly assistance and interest.

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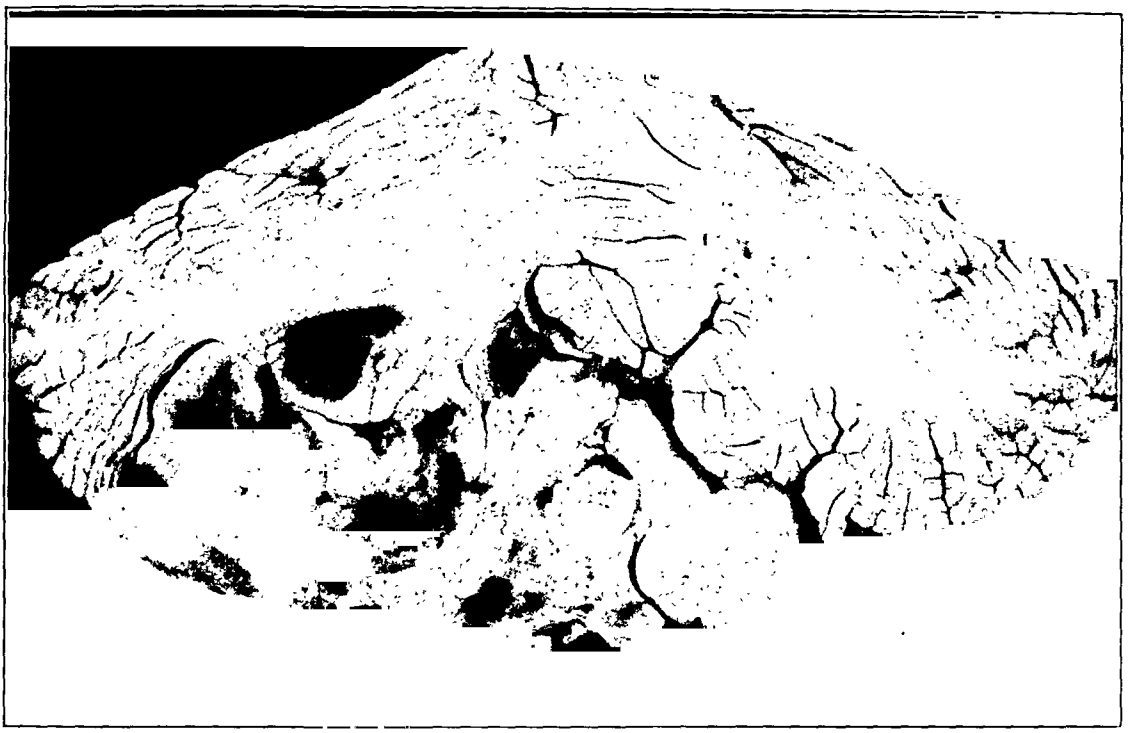
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DESCRIPTION OF PLATES

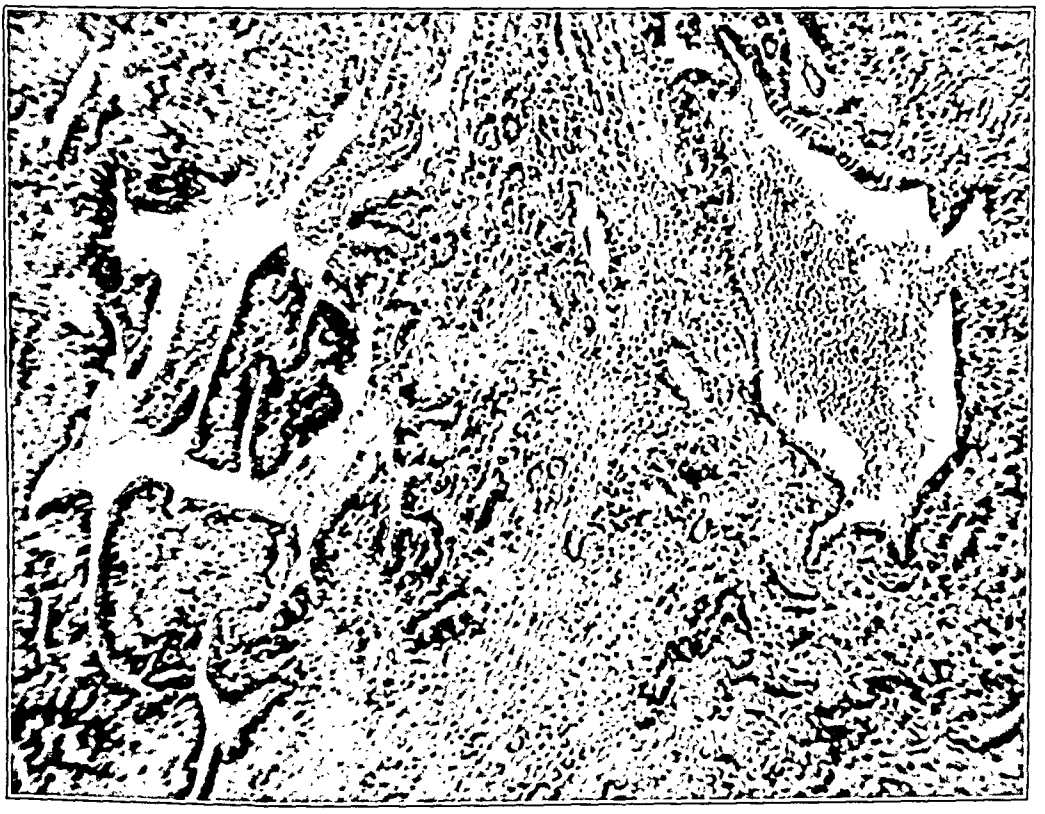
PLATE 32

FIG. 1. Section through the tumor at its center showing its extent, invasion of the cerebellum, and relation to the fourth ventricle and the compression of the pons. The numerous areas of degeneration and hemorrhagic infiltration can also be seen.

FIG. 2. Another portion of the tumor showing large cavities lined by primitive spongioblasts. The walls of the cavities are thrown into folds. The cavity on the right contains many erythrocytes, but is obviously not a blood vessel. Many rosettes can be seen in the intervening tissue. Hematoxylin and eosin.



1



2

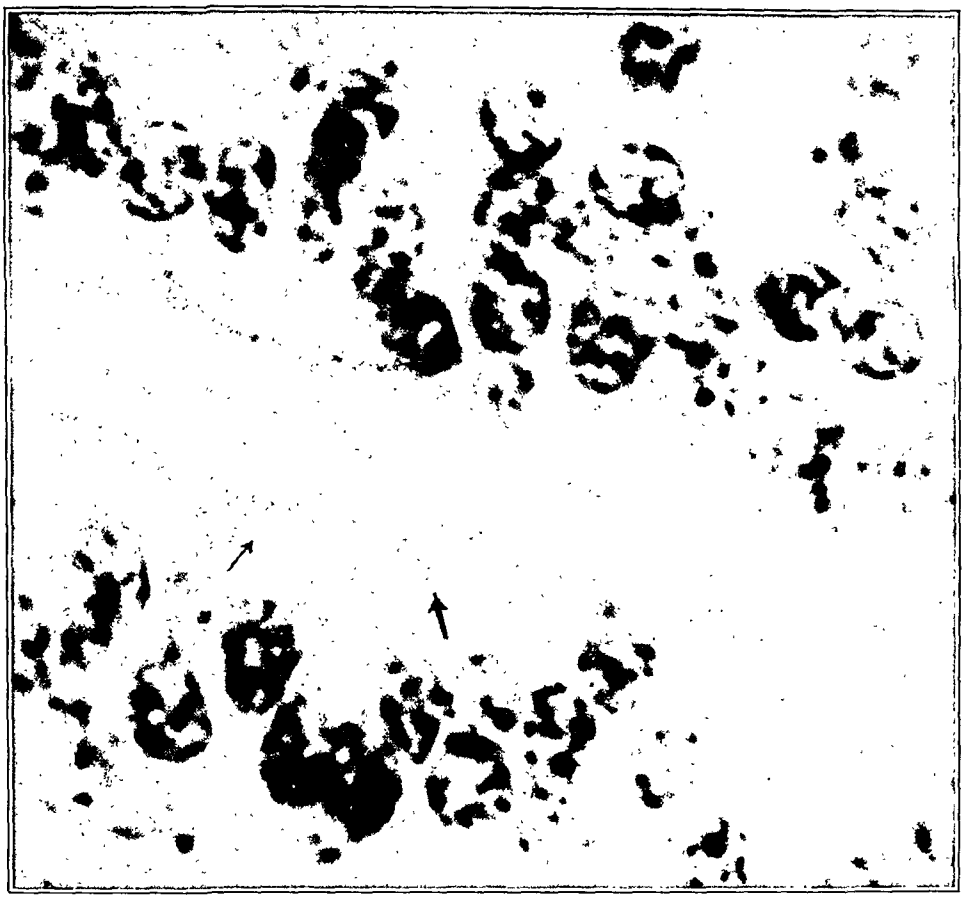
Bucy and Muncie Neuroepithelioma of Cerebellum

PLATE 33

FIG. 3. Single rosette showing blepharoplasten at the inner ends of the cells. Mallory's phosphotungstic acid hematoxylin. Very high magnification.

FIG. 4. Characteristic appearance of the tumor at its periphery, showing numerous rosettes. Hematoxylin and eosin. Low magnification.

FIG. 5. Rosettes at higher magnification than in Fig. 4. The absence of a limiting membrane, and the cuticular lining of the canals are characteristic features. Note that the peripheral extensions of the high columnar cells forming the rosettes do not stain. Mallory's phosphotungstic acid hematoxylin.



3



4



5

Bucy and Muncie

Neuroepithelioma of Cerebellum

PLATE 34

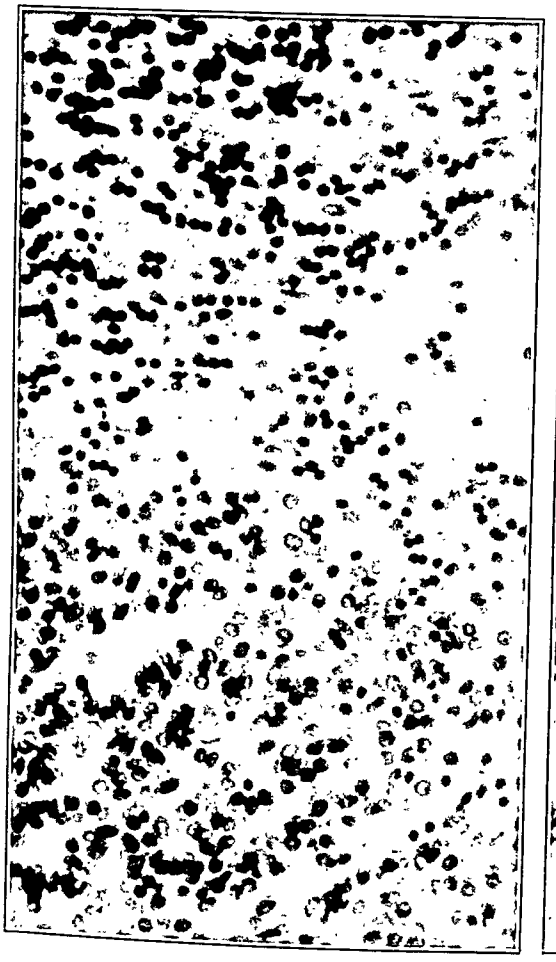
FIG. 6. Photomicrograph taken at the edge of a rosette, the edge of the canal lies at the right margin, with blood vessels in the center of the field. The tails of the cells which are shown very clearly can be seen running from the cells about the canal to the neighboring blood vessels. Cajal technic for non-medullated nerve fibers, overstained, non-specific. × 800.

FIG. 7. Junction of the cerebellum and the tumor. The intermingling of the lightly staining indifferent cells of the tumor below with the darkly staining smaller cells of the granular layer of the cerebellum above is well shown. The tumor cells at this point show no tendency toward organization. Their cytoplasm is very indefinite. Hematoxylin and eosin.

FIG. 8. Margin of an area of massive degeneration from the center of the tumor. A few cystic cavities can be seen on the left. Hematoxylin and eosin.



6



7

Bucy and Muncie



8

Neuroepithelioma of Cerebellum

THE EFFECT OF COMBINED FEEDING OF POTASSIUM IODIDE AND ANTERIOR LOBE OF THE PITUITARY UPON THE THYROID GLAND *

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In a series of studies on compensatory hypertrophy of the thyroid gland Loeb [1] has shown that the oral administration of potassium iodide in partially thyroidectomized animals not only fails to prevent hypertrophy but may even stimulate regenerative activity, while the feeding of anterior lobe of pituitary gland has an inhibiting effect preventing even moderate degrees of hypertrophy and causes a hardening of the colloid and a lowering of the acinus cells. The effect of potassium iodide on the normal intact thyroid has also recently been studied and a method evolved for determining the proliferative activity in a definite and quantitative manner by estimating the number of mitoses in the gland at any given time.[2, 3] By this means the stimulating effect of iodine on mitotic proliferation in the thyroid has been established.

In view of the antagonistic action of these two substances on thyroid activity in compensatory hypertrophy, it seemed interesting to see if the stimulating effect of potassium iodide would counteract the inhibiting action of anterior pituitary substance, or vice versa, when both are fed to the same animal. If these two substances completely neutralized each other, as far as their influence on the thyroid is concerned, it should remain unaltered, but if one were dominant the degree of its influence could be ascertained by estimating the mitotic activity of the gland.

The experiments to be described concern the effect of such combined feeding with potassium iodide and anterior pituitary on the thyroid gland; the result of administering each of these two substances separately to different animals and a comparison of these three types with normal thyroids from control guinea pigs.

Three series of experiments were carried out. Guinea pigs were used and in each series there were four groups: (*a*) animals fed with

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KI, each receiving a daily dose of 0.05 grams, made up in a pill; (*b*) those fed one 5 grain tablet of anterior pituitary (Armour & Co.) every day; (*c*) others receiving daily both the 5 grain pituitary tablet and the 0.05 gram pill of KI; and (*d*) control animals. The four groups of each series were kept under as nearly identical conditions as possible. All received the same kind of food and at the end of the experiment were killed with chloroform and the thyroid glands were fixed in Zenker's fluid. Serial sections were made of all the thyroids.

The number of mitoses in each gland was computed by counting those in every tenth section. This estimate constitutes a reliable index of mitotic proliferation in the thyroid gland. In studying the sections the size of the acini, the character of the cells and the conditions of the colloid were compared.

I. The Number of Mitoses

Animals Fed with KI: In the first series five guinea pigs were fed KI for twenty days and showed an average of 498 mitoses in the whole thyroid. The number of cells in division in this group was less than had been encountered in previous experiments in this laboratory. This difference could be partly accounted for by a slight loss in weight sustained by some of the animals during the course of the experiment, for it has been observed (by Loeb and by Rabinovitch) that the degree of compensatory hypertrophy diminishes and that potassium iodide fails to call forth the maximum karyokinetic response during periods of underfeeding when the animals are losing weight. However, some of these guinea pigs gained in weight and still showed lower counts than one would expect from the histological character of the sections, suggesting that perhaps this batch of animals happened to be more sensitive to KI and that therefore at twenty days the period of maximum activity had already passed and the thyroid was then approaching the next stage in which mitotic activity shows a noticeable decline. Because of this experience, in the second and third series the animals were fed KI for a period of only fifteen days. The average number of mitoses for both these groups was 1919.

Pituitary-Fed Animals: Altogether ten animals were fed each a 5 grain tablet of anterior pituitary daily. In five, the feeding extended over a period of twenty days and in five, for a period of

TABLE I

First Series

Potassium Iodide Feeding		Pituitary Feeding	
Guinea pig	No. of Mitoses	Guinea pig	No. of Mitoses
1	370	11	35
2	568	12	56
3	392	13	21
4	460	14	48
5	700	15	70
Average	498	Average	46

Second Series

6	1710	16	74
7	1697	17	55

Third Series

8	2130	18	80
9	2250	19	107
10	1810	20	76
Average	1919	Average	78
Average for all three series	1208.	Average for all three series	62.

First Series

Potassium Iodide and Pituitary Feeding		Controls	
21	Died	33	85
22	"	34	130
23	"	35	92
24	150	36	196
25	214		
Average	182	Average	125

Second Series

26	142	37	170
27	160	38	60
28	121	39	40
29	80		
Average	125	Average	90

Third Series

30	96
31	130
32	110
Average	112
Average for all three series	133
Average for all controls	110

fifteen days. The average number of mitoses was 62 for these ten animals.

Combined Feeding with KI and Anterior Pituitary: The average number of mitoses in this group, comprising nine animals, was 133. In addition there were three animals in the first series which died before the twentieth day and were not included in this list.

Control Animals: In the control animals the average number of mitoses was 110 per gland. A summary of the mitotic counts will be found in Table I.

II. Effects of KI and Pituitary Separately and in Combination on the Structure of the Thyroid Gland

Control Animals: The normal guinea pig thyroid is composed of small to medium-sized acini, the largest ones usually being situated at the center of the gland. Three zones of acini can usually be distinguished; the peripheral and central areas contain large acini and the intervening zone is made up of smaller acini. Low cuboidal epithelial cells with prominent nuclei line the acini that contain solid, homogeneus, intensely staining, slightly retracted colloid. There is, however, considerable variation in structure in different animals. The size of the acini may vary and at times the colloid is somewhat softer, staining less intensely with eosin and showing vacuoles in the peripheral zone. Phagocytic cells are now and then seen in the colloid, though this is a rare occurrence in normal glands. In older guinea pigs collections of lymphocytes may occasionally be found in the connective tissue of the stroma.

Potassium Iodide-Fed Animals: The histological appearance of thyroids from animals fed with KI has been dealt with at length in papers by Gray [2] and Loeb and Rabinovitch.[3] The changes in the thyroids of this group are essentially the same as those described by them. Briefly, the changes in the first series that were fed for twenty days are as follows: The acini are larger than in the controls and, at first glance, their epithelium seems on the whole more flattened than normally, except where there is mitotic division. This apparent flattening, however, is evidently due to the pressure exerted on the epithelium by the increased content of the acini and one can readily see that in places where no pressure is exerted on the acinar cells they are larger than normally, and even in large distended acini

where they are spread out to cover a larger surface their volume is unquestionably greater than that of the same number of cells in control animals. A feature concomitant with these changes is a great reduction in the amount of solid colloid, it being replaced by liquefied colloid which either stains very lightly or does not stain at all. Under low power the great variation in the amount of solid and liquefied colloid in the different acini is very striking. Groups of phagocytic cells are found in the colloid within many of the acini and form a very marked feature in this twenty-day series.

The changes observed in the two fifteen-day series are not as obvious as those just described. The size of the acini and character of the colloid do not differ much from the controls. The epithelial cells, however, are somewhat hypertrophied and increased in number. They also show many mitotic figures. The number of phagocytes in the colloid is not as great as in the twenty-day series.

Anterior Pituitary-Fed Animals: Thyroids of this group show acini crowded closely together and completely filled with a very solid intensely staining and non-vacuolar colloid. The absence of retraction of the colloid from the lining cells is a distinctive feature of the pituitary-fed animals. Everywhere the epithelium is very low as if compressed by the colloid in the distended acini. At the center of the gland an occasional large acinus has a slightly softened, pale, bluish colloid, and it is in the epithelium of these that most of the remaining mitotic figures are found. The acini in this group are also on the whole noticeably smaller than in the controls.

Combined Feeding with KI and Anterior Pituitary: Thyroids from these animals show certain variations depending upon the length of feeding. Those fed for twenty days (only two animals) have a central zone composed of a small number of rather large acini some of which contain a soft, light-staining colloid. In some of these acini a few phagocytic cells are found, though they are not numerous. The rest of the thyroid about this central zone is composed of small to medium-sized acini distended with a dense solid colloid extending to the cells but not compressing them as in the pituitary animals. In this outer zone the acini are close together but are not often crowded sufficiently to obscure all traces of the loose connective tissue stroma seen in normal glands.

Those animals fed for only fifteen days show essentially the same changes except that the central zone of large acini with softened

colloid is absent and acini with solid colloid are seen throughout the gland. Even in this series an occasional acinus with pale-staining colloid is found in the center of the thyroid but phagocytic cells are entirely lacking. The acini are small and are lined by a low to medium-sized epithelium but are not as noticeably compressed or flattened as in the twenty-day series. The acini are in some places close together but for the most part are slightly separated by thin strands of connective tissue.

The appearance of these thyroids stands midway between those from normal and those from pituitary-fed animals. Their acini are about the same size as in the normal gland but are separated by less stroma. They are, however, not as crowded as in the pituitary animals. The epithelium is slightly flattened but not compressed into a thin strand of cells as is often seen in the pituitary group. The colloid is hard, stains intensely with eosin and completely fills the acini; it is not vacuolar and is uniform in amount, thus differing from the normal.

These glands then show the influence of anterior pituitary substance. It might be possible to so balance the dosage of these two substances that the thyroids would retain their normal characteristics and not approximate the condition found in animals fed either with KI or with anterior pituitary gland.

Discussion

From these experiments it may be concluded that the marked mitotic proliferation induced so readily in the thyroid of guinea pigs by potassium iodide is prevented by anterior pituitary gland when both are fed to the same animal. These two substances administered separately are antithetical in their action on the thyroid; anterior pituitary gland depressing proliferative and probably also functional activity as indicated by a decided drop in the mitotic count below the normal level and by the appearance of small acini filled with a dense deeply staining colloid, while KI causes marked stimulation, in the initial stage of its action at least, with enormous increase in the number of mitoses and with the appearance of a softened, pale, vacuolar colloid containing phagocytic cells. The condition of the thyroid which follows feeding of both these substances to the same animal is due to their direct action on the gland

itself, and not an effect which they exert upon each other before absorption from the gastro-intestinal tract, because changes in the thyroid are the same whether both drugs are fed simultaneously or whether one is fed in the morning and the other in the evening precluding any chance of direct interaction between them.

The stimulating influence of iodine and depressing property of anterior pituitary substance can be balanced in such a manner that an approximately stationary or resting condition in the thyroid results. In some respects anterior pituitary substance produces changes in the thyroid gland analogous to those induced by thyroid itself or by thyroxin. Loeb has shown that thyroid as well as pituitary substance prevents the compensatory hypertrophy that follows extirpation of a major portion of the thyroid gland, leaving open the question as to what constituent of the preparation of anterior pituitary (Armour & Co.) is responsible for this effect.

From these experiments it is impossible to determine the exact manner in which pituitary substance exerts its influence upon the thyroid gland. All that one can say is that anterior pituitary hormone in some way depresses the activity of the thyroid and holds in abeyance its capacity for responding to the stimulating effect of potassium iodide.

Summary

Oral administration of anterior pituitary tablets (Armour & Co.) causes a depression in the activity of the thyroid with a marked lowering of the number of mitoses in the entire gland and with medium-sized, or somewhat smaller, acini distended with hard colloid compressing the lining epithelium into thin strands of cells.

During the first stage of its action, potassium iodide, on the other hand, produces marked stimulation with enormous mitotic activity and a slightly softened colloid occasionally containing large numbers of phagocytic cells.

The early proliferative change induced in the thyroid gland by potassium iodide is prevented by anterior pituitary when both these substances are fed to the same animal.

Note: The author is indebted to Professor Loeb for suggesting this problem and for advice and assistance during its preparation.

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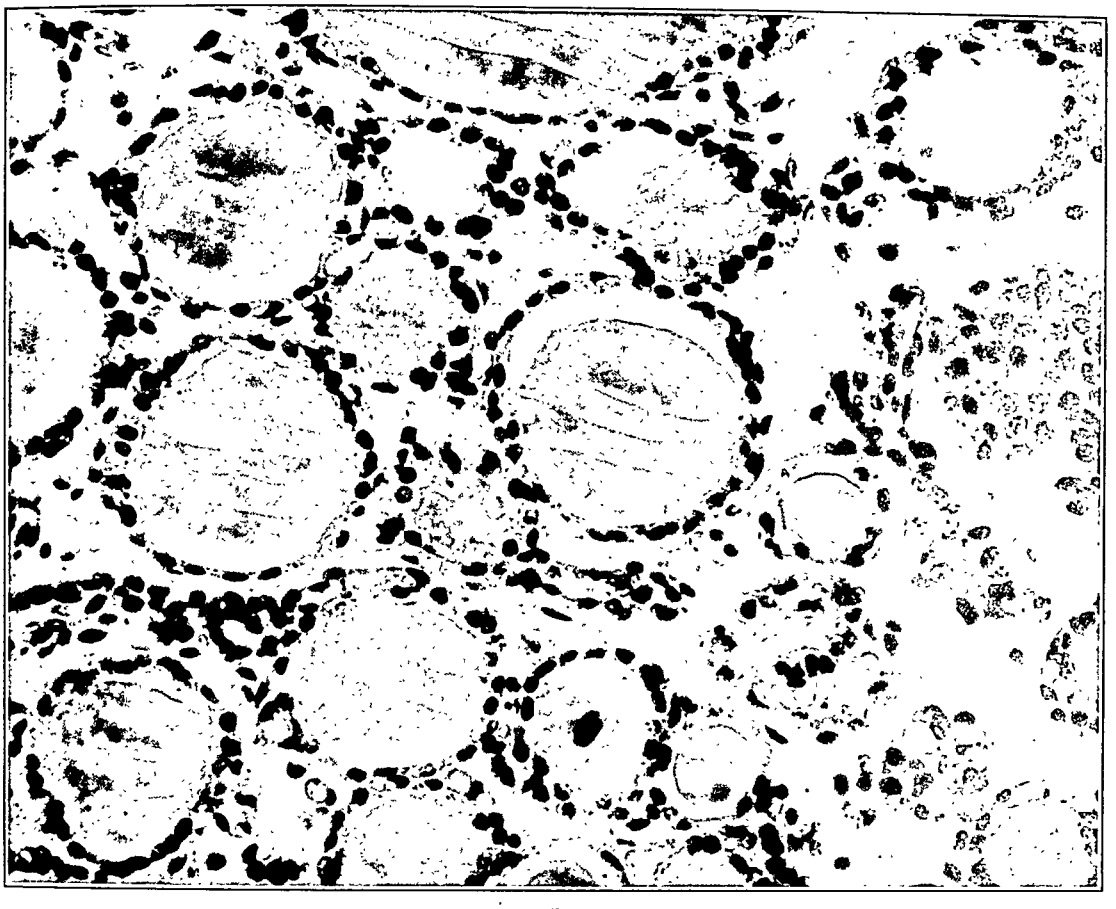
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DESCRIPTION OF PLATES

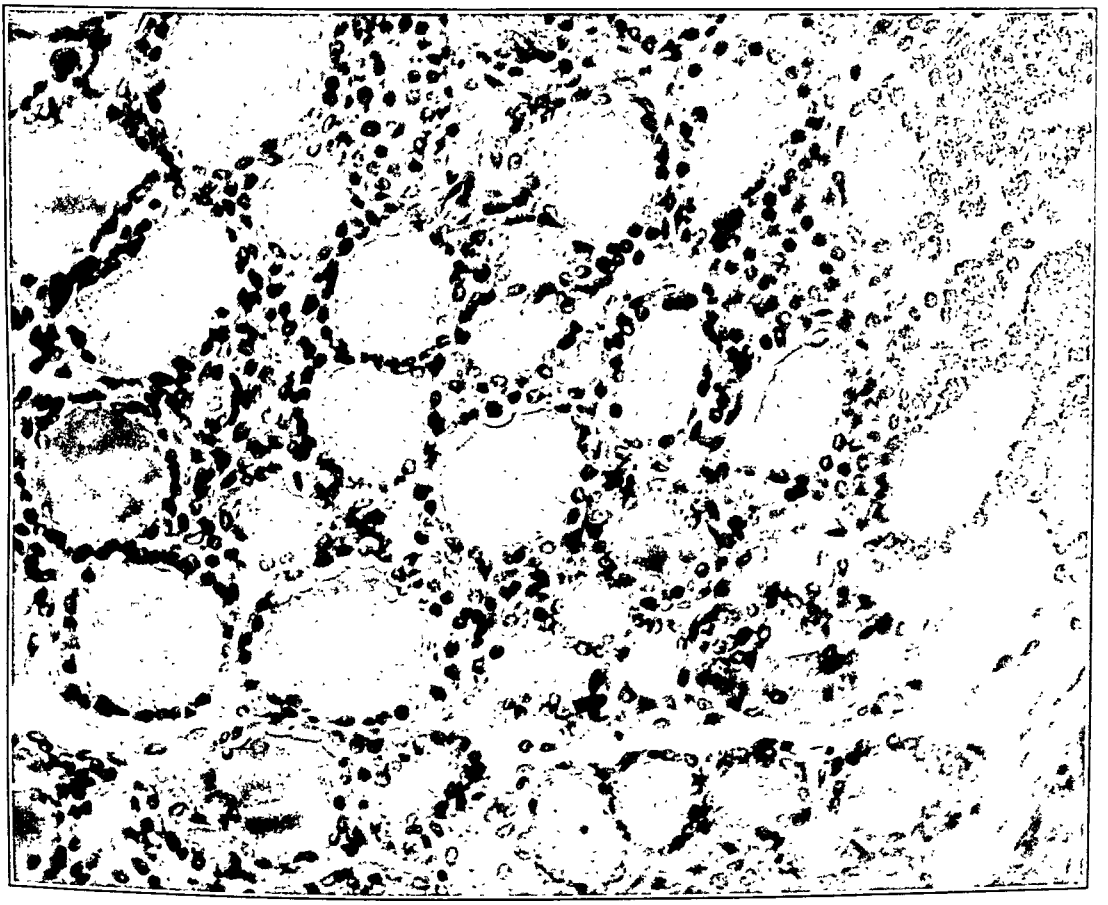
PLATE 35

FIG. 1. Normal thyroid. × 280

FIG. 2. Fifteen days feeding with KI. Several mitotic figures are present near the center of the picture. × 280



1



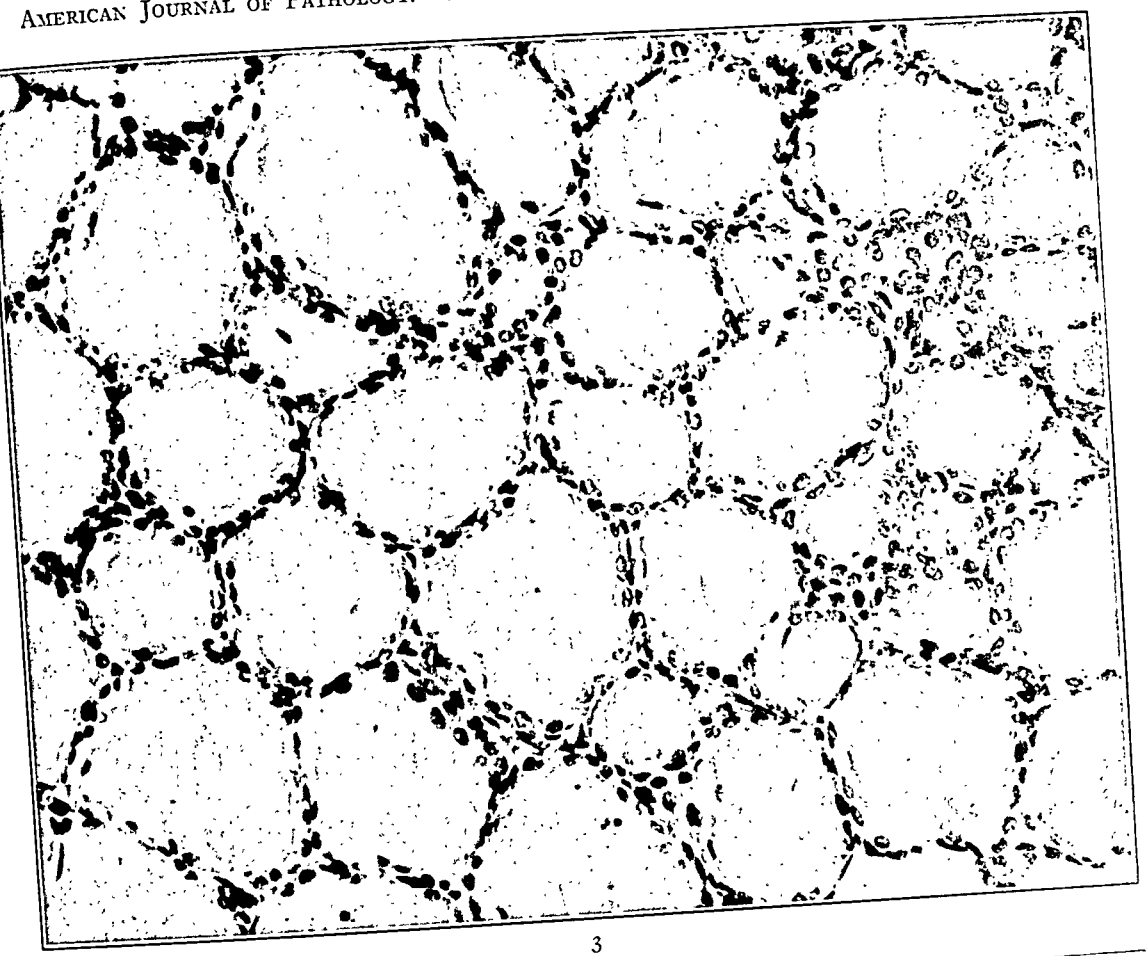
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McCordock Thyroid Gland

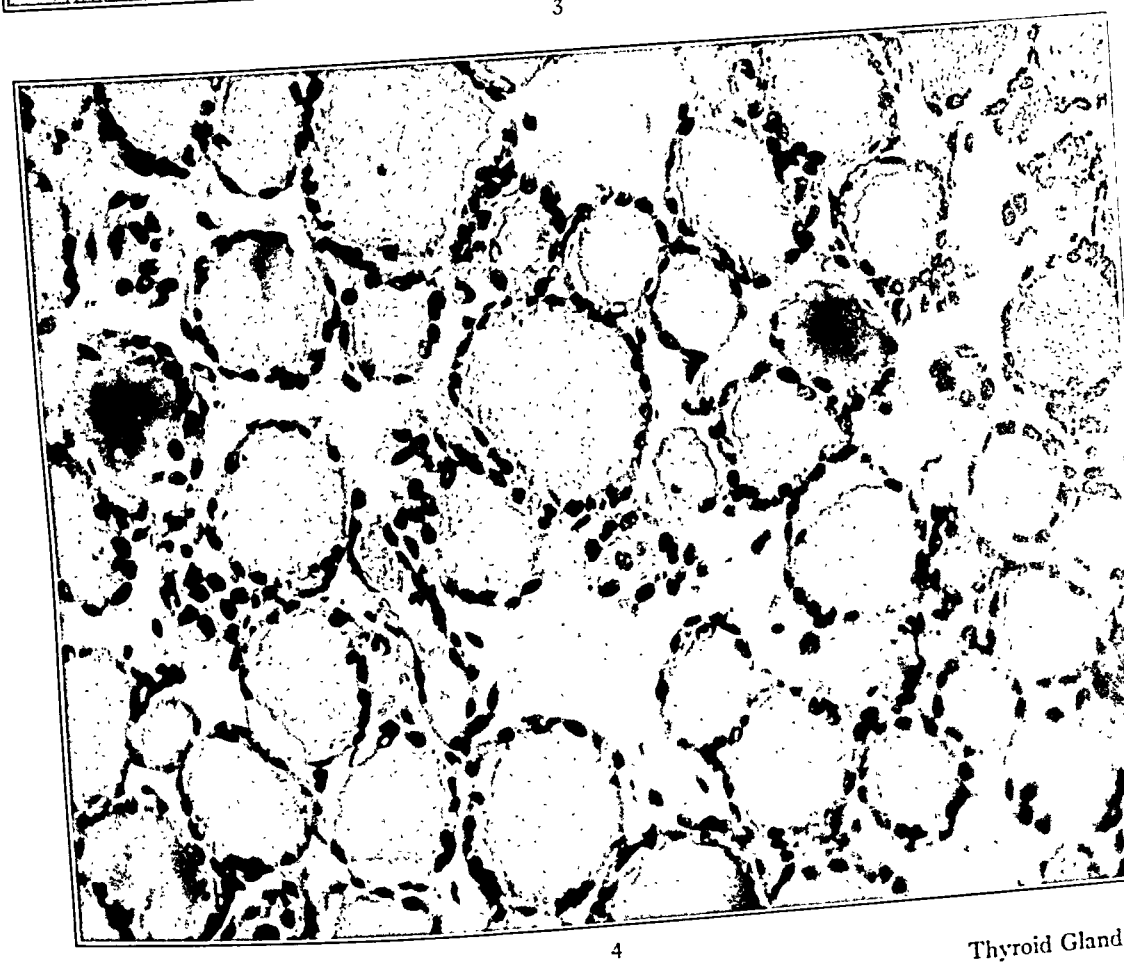
PLATE 36

FIG. 3. Thyroid from animal fed anterior lobe of pituitary gland for fifteen days. × 280

FIG. 4. Combined feeding with KI and pituitary, fifteen days. × 280



3



4

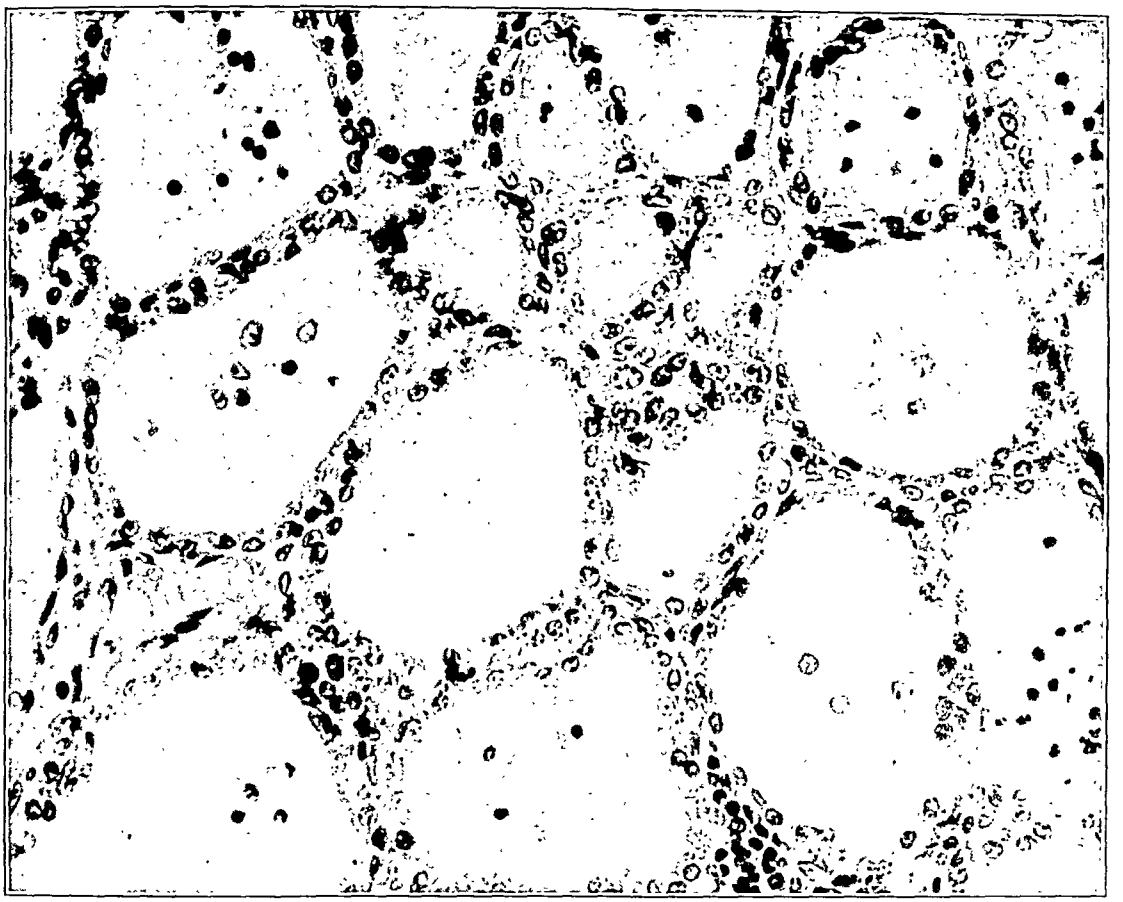
Thyroid Gland

McCordock

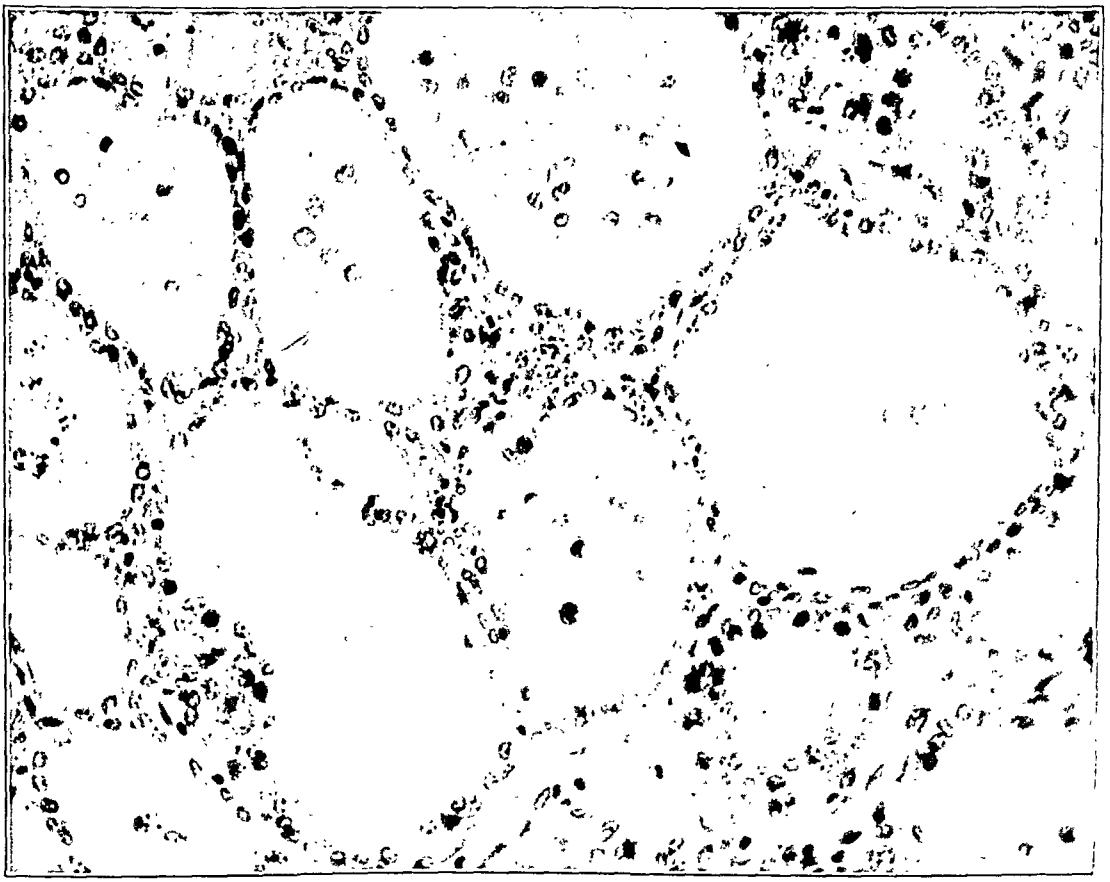
PLATE 37

FIG. 5. KI feeding for twenty days. Compare with Fig. 2. Note large acini with soft colloid containing phagocytic cells and absence of mitoses and hypertrophic epithelium. × 300

FIG. 6. From same animal as Fig. 5. Note large acini. One in lower left-hand corner shows a constriction at the middle indicating the manner in which these large acini are formed by coalescence of two smaller ones. × 300



5



6

McCordock Thyroid Gland

FENESTRATIONS OF THE SEMILUNAR VALVES*

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The following is largely a statistical study of fenestrations of the semilunar valves of the heart in 300 successively observed hearts. With but few exceptions, the specimens were examined at the autopsy table.

The literature of this subject is scant and to my knowledge there are no numerical, statistical studies. From a review of textbooks of cardiology and anatomy, it is easily apparent that there is much confusion as to the nature and frequency of these valve defects. It seems to be generally accepted that they are of no clinical significance.

FREQUENCY

(*a*) There were one or more fenestrations in 82 per cent of all cases.

In 53	cases	no	cusps	were	involved
" 71	"	1	cusp	was	"
" 65	"	2	cusps	were	"
" 39	"	3	"	"	"
" 32	"	4	"	"	"
" 30	"	5	"	"	"
" 10	"	6	"	"	"

(*b*) The aortic and pulmonic valves were involved with a negligible difference in frequency.

Aortic	in 188 cases
Pulmonic	in 187 cases

(*c*) In an estimation of the total number of fenestrations the frequency of the pulmonic involvement exceeded the aortic (pulmonic 1045, aortic 789); whereas in an estimation of the total number of cusps involved the reverse was true (pulmonic 314, aortic 325).

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Age

The age groups, average number of fenestrations per case and age frequency of patent foramen ovale are given in the following table:

Age Group	Number of Cases	Number of Fenestrations per Case	Per cent Patent Foramen Ovale
Fetus	3	1.3	66
2 years and less	20	.7	60
2–10 years	19	4.1	37
2nd decade	6	3.3	0
3rd "	25	5.9	12
4th "	51	8.0	22
5th "	82	6.3	13
6th "	62	7.0	21
7th "	29	7.8	10
8th "	3	2.5	33

It is evident that the frequency of fenestrations increases with age from the fetus to the fourth decade. After this there is a slight decrease, possibly due to the increasing frequency of sclerotic changes which obliterate small defects.

Of the cases under 10 years of age (42 cases), 40 per cent had no fenestrations. Of those over 10 years of age (258 cases), 14 per cent had no fenestrations.

There is no evidence to prove that fenestrations are developmental defects. It is even questionable whether there is any cause in an inherent endocardial or connective tissue weakness. The fenestrations form a pathology fundamentally mechanical in origin. Those few present at birth may be acquired from the mechanical effects of the intra-uterine fetal circulation. On the other hand, the frequency of patent foramen ovale, a distinct developmental defect, decreases with age and especially so after the first few years of life. This decrease may be due to later closure or to association with other developmental defects that lead to early death. In statistical studies of the frequency of patent foramen ovale it is essential that age groups be given. In this series of cases the frequency of this defect was 22 per cent.

It is interesting to note that in the three cases that showed marked developmental defects (bicuspid pulmonic valve, quadricuspid pulmonic valve, interventricular defects), there were no fenestrations; however, the latter two of the three cases had a patent foramen ovale.

Sex

In the female the valve leaflets are less frequently involved. In males, aortic, pulmonic, or both valves were uninvolved in 55 per cent; in females, in 64 per cent.

Etiology and Classification

Mechanical: (*a*) Circulatory (persistent strain of current).
(*b*) Traumatic.
(*c*) Ulcerative (infection plus strain of current).

Apparently the most frequent cause is the persistent strain of the blood current against the valve leaflets. It is questionable whether the perforations of ulcerative endocarditis or trauma (one case of each in this series, the latter due to a bullet wound) should be included in a classification of fenestrations. An understanding that the defects are all fundamentally mechanical in origin would justify such a classification.

Nature and Variety of the Fenestrations

The most frequent site of the fenestrations is adjacent to the attachment of the free edge of the cusp to the aortic intima. The fenestrations are usually ovoid apertures, with the long axis parallel to the free edge of the valve, and are apparently defects [1] in the endocardium that bridges the spaces between the connective tissue strands of the valve stroma. The defects may be single or multiple and may form large gaps in the valve surface. Across these gaps the remnant strands of fibrous tissue cross and intersect each other to form a veritable network which at times has a peculiar anatomic beauty.

The fenestrations occur less frequently along the free edge of the valve, and then as a rule well away from the corpora arantii. Due to adhesions of the free edges of two cusps the fenestration may permit no communication with the heart cavity, but form, instead, a passage between two sinuses of Valsalva.

Very large numbers of fenestrations are rare. In one case, a man aged 25, the aortic valve had 36 and the pulmonic valve 26 fenestrations. All of the leaflets were involved. Rarely the fenestrations are so fine as to defy count with the naked eye. This occurs in valves that have become markedly sclerotic and thickened.

Clinical Significance

Clinical data were lacking in so many cases that it was impossible to correlate any of the findings with murmurs, blood pressure readings, etc. In two cases the fenestrations were so large and numerous as to bring to mind the possibility of an aortic insufficiency. In one of these, with a non-dilated aortic orifice the fenestrations occupied at least one-half the valve area.

Summary

1. In a series of 300 cases one or more fenestrations occurred with a frequency of 82 per cent.

2. The frequency of pulmonic and aortic involvement was approximately equal.

3. Fenestrations, which are acquired defects, increase in frequency with age.

4. Patent foramen ovale, which is a developmental defect, decreases in frequency with age. The total percentage for the frequency of the occurrence of this defect was 22.

5. Classification, nature and variety of the fenestrations are briefly discussed.

6. It is possible that fenestrations may occasionally cause an aortic insufficiency.

My thanks are due Dr. Charles Norris, Chief Medical Examiner, and Dr. Douglas Symmers, Director of Laboratories at the Bellevue Hospital, for permission to study and report these cases.

Note: Since the submission of this paper for publication I have observed a large perforation of one of the aortic leaflets, apparently due to an atherosclerotic ulcerative process. Interesting also are the experimental perforations of the semilunar valves in animals. These were followed by evidences of aortic insufficiency (Herrmann, G. R. *Am. Heart J.*, 1926, i, 671).

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SO-CALLED INFECTIOUS SARCOMA OF THE DOG IN AN UNUSUAL ANATOMIC SITUATION *

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Few animal tumors have been the subject of more investigational study than the so-called infectious sarcoma of dogs. This growth is also known to veterinarians as "venereal granuloma" and as "venereal lymphosarcoma." The disease is not infrequently seen in kennels which house large numbers of dogs, and it occasionally appears in individual dogs not associated with kennel life. Practically all who have studied the condition agree that the primary focus of the disease is either the penis of the male or the vagina of the female, and that the disease is transferred to unaffected, susceptible animals by affected animals during the act of coitus.

Although the literature of the subject is known to many, it would seem apropos, in view of the unique site of the tumors in the case which constitutes the basis of this paper, to give a brief summary of the more important published reports concerning this interesting tumor.

Nowinsky [1] is given credit for the first published observations on this tumor. He reported a transplantable neoplasm which was removed from the vagina of a bitch and which recurred after a period of three months. He diagnosed the tumor myxosarcoma, and it was successfully transplanted subcutaneously into three puppies, aged eight days, fourteen days, and two months respectively. Metastasis was not observed.

Wehr [2] reported successful transplantation of the tumor which he considered a carcinoma. Geissler [3] also considered the growth to be a carcinoma, although Hansemann discussing Geissler's paper was of the opinion that the tumor was not a carcinoma.

Duplay and Cazin [4] were the first to suggest the possible inflammatory nature of the growth, although in one of the animals which bore successful transplantation of the tumor, metastasis to one testicle was noted. Bashford, Murray and Cramer,[5] after a careful study of

* Received for publication January 18, 1929.

the disease denied that it was neoplastic and, although they admitted that there was histologic resemblance between this growth and round-cell sarcoma, they concluded that "these tumors of the vagina and penis of the dog are connective tissue reactions to a living virus which has not yet been demonstrated."

In a series of papers dealing with this tumor, Smith and Washbourn [6, 7, 8] described an instance in which twelve bitches were served by a dog affected with a tumor on the penis. Vaginal tumors developed in eleven of the bitches. Three of the bitches which possessed transplanted vaginal growths were served by a second male on whose penis a tumor developed subsequently. The second male in turn served two unaffected bitches and a vaginal tumor developed in one. These authors also recorded a case in which a healthy dog was permitted to serve a bitch with a vaginal tumor and, in spite of the fact that the penis was frequently washed after coitus, a number of small growths developed on the part. They noted that transplanted growths were visible within one week after inoculation as small "glistening elevations about the size of millet seeds, mostly transparent but sometimes blood-stained. They looked like vesicles, but when they were pricked they proved to be solid." In three instances, Smith and Washbourn found the inguinal lymph nodes invaded with metastatic tumor cells, and in one dog metastasis was present in the liver and the spleen. They considered that the highly bred and more sensitive breeds, and dogs in poor general condition, offered more favorable environmental conditions for the growth of the tumor than were offered by the mongrel type or those that are well nourished. They noted spontaneous recovery with immunity to subsequent exposures in several instances.

From the results obtained in a series of inoculation experiments and from a general study of the disease, Smith and Washbourn noted that death may occur from "cachexia or from kidney disease, the result of obstruction of the ureters or from septic poisoning owing to sloughing of the growth." They concluded: (1) the tumors are infective round cell sarcoma; (2) they can be transplanted from the genitals, where they naturally occur, to the subcutaneous tissues of dogs; (3) they can be transplanted from the subcutaneous transplants in like manner through a series of dogs; (4) their spontaneous disappearance may occur with or without ulceration; (5) death may occur from metastatic deposits of the tumor in the viscera; and (6)

if the tumor disappears, the animal is then immune to subsequent inoculation.

Sticker,[9, 10, 11] who considered the growth to be a round cell sarcoma, made several important observations concerning its physiologic and pathologic aspects. He noted that successful transplants were possible in the spleen, bone and peritoneum, as well as in the skin. He was able also to secure successful transplants in two of three foxes which were used, but he was unsuccessful with cats, rabbits, guinea pigs and mice. In 16 per cent of all dogs with transplanted tumors in Sticker's series, the growths disappeared spontaneously, and these animals were immune to reinoculation. Sticker experimented with the viability of the tumor cells and found them quite resistant to extremes of temperature. They remained viable after twenty-four hours of exposure to a temperature of -14° C, and after two hours of exposure to 50° C. On the other hand, attempts failed to produce growths from cells which were kept at -11° C for twenty-five days and from cells which had been subjected to crushing. Neither did growths result from filtered or centrifuged emulsions of cells.

White's[12] observations concerning the contagiousness and malignancy of the tumor are of interest. Two pure-bred bull bitches were served by an apparently healthy male, and vaginal tumors developed in both shortly after whelping. In one of the dogs, a mammary growth also appeared. Both bitches were operated on and after removal of the tumors they recovered completely. They remained well, were bred twice afterward, and each gave birth to normal litters. The male used to breed the first two bitches also served four other females, in all of which the growths developed and they died in consequence of them. One of the first two bitches was served by three other males, and all died of the disease.

White believed that these growths have several features in common with infectious diseases, but he concluded that the growths which occur following inoculation have their origin from the transplanted cells and not from a separate infective agent. White also pointed out certain differences between the tumor and the usual forms of sarcoma. He mentioned the marked contagiousness of the venereal growths and the relatively mild malignancy of the majority of these tumors in that they show little tendency to infiltrate the surrounding tissues and do not often produce metastasis.

Beebe and Ewing [13] undertook to show by experiments with transplantation the exact manner of origin of the tumors which resulted. They transplanted small grafts of tumorous tissue subcutaneously and made histologic studies of excised portions at intervals of one to twenty-one days. This was done in an attempt to determine "whether the growth comes from the proliferation of the transplanted cells or by stimulation of the surrounding tissue cells as maintained by Bashford, Murray and Cramer." Beebe and Ewing found that in transplants all the tumor cells do not die; some at the periphery remain alive. These undergo mitosis and "leave no doubt that the transplanted cells give rise to the tumor." Concerning the nature of the disease, these writers were "forced to conclude that the infectious lymphosarcoma of dogs is a true malignant neoplasm." They admitted, however, their inability to determine the exact tissue cells involved in the histogenesis of the tumor. At the time of Beebe and Ewing's report, the tumor with which they worked was in its fifth generation, and thirty-five tumors had developed in the twenty-nine dogs used. Their paper includes a rather complete description of the clinical aspects of the disease, and they stated that in both sexes isolated tumors may occur anywhere over the body as well as in the spleen, kidney, liver and lung.

Wade [14] also conducted a rather exhaustive investigation of the disease during the course of which he successfully inoculated two fox cubs. The animals died, much emaciated, after the tumors had attained the size of about 2 by 1 cm.

Rabbits, rats and guinea pigs resisted successful transplantation of the tumor, and six dogs that had recovered spontaneously from previous successful inoculations apparently were immune to attempts at reinoculation. Wade observed interstitial nephritis in many successfully inoculated dogs, and he thought this lesion represented the effect of a soluble toxin from the tumor cells and constituted the strongest evidence advanced up to that time in favor of the disease being an infective granuloma and not a true neoplasm. It was Wade's opinion that the growths which occurred as a result of inoculations of tissue had their origin in part from the vigorously dividing peripheral cells of the tissue which was introduced, and in part from the surrounding connective tissue fibroblasts which become altered in the process of repair and assume an immature sarcomatous nature. He therefore considered the growth to be an

infective sarcoma, a tumor belonging "to the borderline between the infective granulomas and the true neoplasms." Those tumors which arise after natural infection Wade believed to consist of cells derived entirely from the tissue of the host.

In another report by Beebe,[15] the correctness of considering these growths to be of an infectious nature was questioned. Beebe felt that evidence in support of the action of microörganisms in their genesis was lacking and brought out the fact that successful transfers of the tumor had been accomplished only by means of a living tumor cell. Beebe again expressed his belief in the true neoplastic nature of the process.

Crile and Beebe[16] subscribed to the view that these growths are neoplastic as follows: "From the total available evidence we are convinced that the process is a true tumor." These workers made some extremely significant observations concerning the curative value of blood from animals that had recovered spontaneously. In brief, they effected direct transfusion of whole blood from an animal that had recovered spontaneously and therefore was immune to an animal with an actively growing tumor. Of ten tumorous dogs that were the recipients of transfused blood, seven were cured, and in two the transfusion apparently produced marked inhibition or regression of the growth. Crile and Beebe did not think the protective or curative properties that were exhibited were analogous to bacterial immunity; nevertheless, they thought that there was present a specific factor capable of rendering an affected animal passively immune.

Two other papers that have to do with the use of certain biologic products in the treatment of these tumors were written by Bergell and Sticker,[17] and by Beebe and Tracy.[18] Bergell and Sticker were able to cause complete disappearance of the tumor, in cases in which spontaneous recovery was unlikely, by injection of a specific hepatic ferment. In one case, with metastasis to lymph nodes, injection of the hepatic ferment caused regression which extended to the involved node. Following the injection, the tumors became cystic with marked phagocytic action by macrophages, and, in place of the numerous mitotic figures which characterize the tumor in its progressive stage, the injected tumor was quite devoid of mitotic figures. Beebe and Tracy demonstrated the destructive influence of certain bacterial toxins on the tumor. Tumors at a distance from the in-

jected growth regressed simultaneously with those injected. In one instance, an animal with four transplanted tumors received four injections at some distance from the growths, and six weeks after the first injection the tumors had completely disappeared. The authors were not convinced that the effect produced was peculiar to the bacterial toxin used, since they closed by saying, "It seems possible . . . that an equal bulk of any toxic organism would exert a similar destructive action."

Novak and Craig [19] recently reported one case, and after reviewing the literature they concluded that the weight of evidence seemed to substantiate the contention of Beebe and Ewing that these growths are true tumors and not inflammatory granulomas.

Attesting to the universal distribution of the disease is the statement of Seligmann [20] that the disease was endemic in New Guinea before the advent of the white man and hence was not dependent for its appearance on imported dogs.

Since the disease as observed by the investigators mentioned involved the genitals, the finding of tumors histologically similar in every detail to the so-called infectious lymphosarcoma, but in regions entirely apart from the genitals, is considered of sufficient importance to justify the report which follows.

Report of Case

The animal was a mongrel female terrier pup of unknown parentage. In the latter part of June 1928 when she was approximately four months of age the right eye appeared to bulge outward. On examination the eye was found to be opaque and of a cream color, and it seemed to be crowded forward by pressure from behind. Soon after this observation a small nodular tumor was noted under the skin of the frontal region, slightly to the left of the median line (Fig. 1). The opaqueness of the eye gradually changed to a bright red and August 22 a lobulated tumorous mass occupied the bulk of the orbital space, and the eyeball could not be seen. The frontal tumor grew rapidly and August 22 it measured 4 cm. in diameter and was raised above the surrounding tissue 3.5 cm. The frontal mass apparently occupied a subcutaneous position without involving the underlying bone. Other tumors were not observed elsewhere over the body.

September 11, 1928, the tumor of the eye, which had been growing vigorously, had become badly infected and for humane reasons it was decided to attempt surgical removal of both tumors (Fig. 2). This was done under general anesthesia. The eye mass was broadly attached to the posterior portion of the orbit, and the eyeball was completely embedded in the more anterior part of the tumor. The growth was very vascular and the resultant hemorrhage was profuse. The tumor in the frontal region was found to lie just under the skin and to be firmly attached to the underlying bone of the skull; this made complete removal difficult.

Grossly, the tumors were of firm texture and of a grayish white color. At the time of the removal of the tumor the mass in the eye seemed more vascular than the frontal tumor, whereas the latter was of firmer consistence.

From the beginning of the disease the general condition of the dog was excellent. She remained frolicsome and apparently had normal bodily growth. Notes on the animal's subsequent condition follow:

September 24. Both wounds are healing satisfactorily; the animal is in good spirits.

October 10. The frontal wound is nearly healed; the orbital wound is discharging a thin, purulent fluid.

October 30. The frontal wound is completely healed. The orbit continues to discharge a thin grayish fluid.

November 27. There is no apparent recurrence in the frontal region. A small amount of fluid is still draining from the eye socket. Recurrence of the growth that involved the eye cannot be demonstrated.

December 26. There is no recurrence of either tumor. The space from which the tumor of the eye was enucleated has healed completely, although a superficial watery discharge persists (Fig. 2).

Immediately after the removal of the tumors from the body and before their exact nature was determined, they, unfortunately, were placed in fixative fluids, and this precluded possible attempts at transplantation, the desirability of which was made evident by subsequent observations. It is regrettable that a portion was not reserved for experiments with transmission since the subsequent diagnosis revealed a type of growth similar in all its morphologic details to the so-called infectious lymphosarcoma (Figs. 3, 4 and 5).

Microscopic Examination

The tissue in the tumor of the eye consisted of rather large irregularly shaped cells inclined to be polyhedral in outline. They were closely packed together and the continuity of the cell masses was interrupted by strands of fibrous tissue of variable thickness which supported the numerous blood vessels of the growth. Aside from the fibrous strands just mentioned, there was no demonstrable stroma supporting the cells of the tumor (Fig. 6). There was much extravasation of blood in some of the fields and necrosis with some edema on the surface of the growth. Some of the larger vessels near the surface were thrombosed. The cytoplasm of the neoplastic cells was finely granular and was moderately acidophilic in staining quality. The nuclei, which represented about half of the cellular bulk, possessed a large number of coarse chromatin granules, and there was marked affinity of the whole structure for the basic stain.

Mitosis was present in many cells in practically every field, and several interesting phases of this phenomenon could be seen. A prominent, slightly eccentrically located nucleolus could be seen in the majority of cells (Figs. 6 and 7).

The cells were markedly invasive, and the tumor had every structural feature characteristic of a highly malignant tumor. Attempted encapsulation or other evidence of resistance on the part of the normal tissues was not apparent.

The elements present in the tumor of the frontal region, and their arrangement, were an exact replica of the tissue which constituted the tumor of the eye (Fig. 5). It was noted, however, that that portion of the tumor nearest the skin was covered with a dense layer of fibrous tissue of considerable thickness, which contained a large number of small vessels. Inflammatory leucocytes were absent from this tissue. Although for the most part the fibrous connective tissue appeared to be of an adult type, a few areas could be seen near the substance of the tumor where the fibroblasts were somewhat less mature. In these areas some large wandering cells were present between the loosely arranged strands of tissue.

From a study of the material from both tumors it was at once apparent that the growths were similar and that they resembled in every detail the tumor of dogs previously designated infectious lymphosarcoma or venereal lymphosarcoma.

Discussion

This case is of interest because the situation of the tumor was unusual. In the literature reviewed only one reference was made to the occurrence of these tumors in regions apart from the genitals. Beebe and Ewing mentioned that isolated tumors may appear anywhere over the body, but they failed to describe such a case. In reply to a communication concerning this point, Ewing [21] wrote as follows: "I have the impression that infectious sarcoma of dogs has been observed in other regions than the genitals. In fact, I think I have seen one case. . . ." Considering the large number of cases studied by the various investigators, and considering that cases of extragenital involvement have not been described, such cases must be extremely rare.

Consideration of the various features of this case leaves many important points unsettled, and opens anew the question of primary

importance in the study of such tumors: What is the exact histogenesis of the type cell constituting these growths?

Lack of knowledge of the parentage of the subject of this report makes it impossible to assemble all the facts one should possess before attempting an hypothesis concerning histogenesis. In this connection, there are at least two points of importance. In view of the facility with which the cells of this particular tumor lend themselves to successful transplantation, the possibility that the tumors in this case are the result of maternal inoculation at the time of birth should be considered. In case the mother possessed a vaginal tumor of this variety, the mass would be subject to considerable trauma at the time of parturition, and it is conceivable that viable cells from the parent tumor could find their way into the natural recesses of the eye, where favorable conditions would permit their multiplication.

The growth of the frontal region might arise in a similar manner, although it is difficult to imagine natural inoculation taking place through the unbroken skin, even through the tender skin of a newborn puppy.

The other possibility of the origin of these growths is that one or both had spontaneous origin from the tissues of the involved parts. Proof is lacking to substantiate origin by inoculation from the vaginal passage, and likewise it is impossible to present acceptable evidence that the tumors originated from cells derived from the tissues of the respective regions. There can be no doubt but that the tissues of the dog are capable, under the proper influences, of producing this peculiar autonomous cell. Whereas the disease usually is the result of the direct transference of living cells from the tumor of one animal to the tissues of the other, the condition must occasionally have a spontaneous origin; otherwise it would not exist. In further support of their non-transplantable origin is the observation of Seligmann that this tumor affected native dogs in New Guinea before the arrival of imported dogs.

There does not appear to be anything peculiar to the tissues of the genitals that would limit the spontaneous origin of this tumor to these regions, and it would seem quite proper to assume that the elements necessary for its inception may and probably do exist elsewhere. Since the tumors so far reported have appeared beneath the mucosa or under the skin, it is possible that the progenitors of the type cell have their origin as part of a natural response to some ex-

traneous stimulus. As far as can be determined, such tumors have not been encountered as primary growths in the tissues of the interior of the body. These tissues are spared much of the injury to which the exterior of the body is subjected. Furthermore, the particularly violent exertions associated with coitus in the dog render both sexes prone to injuries of the genitals.

The multiplicity of the tumors in the case reported resulted from one of two possibilities. Either the growths arose independently of one another or one was a metastatic expression of the other. Whereas, of these two possibilities, it is perhaps easier to accept the idea of metastasis in explanation of the growth, it is not clear which of the two tumors antedated the other. Functional embarrassment was first noted in the eye, although the subcutaneous mass became evident soon afterward. If one of the tumors was the result of metastasis, the extension occurred very early in the growth of the tumor. After they became established, the masses grew with equal facility, but the tumor of the eye was inhibited perhaps by the limiting confines of the orbital space.

All who have studied these tumors have attempted to classify the type cell, and, although a few of the early observers thought the tumor was a carcinoma made up of epithelial cells, the opinion of the majority is that the growth should be considered a sarcoma.

If it is a tumor derived from cells of the lymphocytic series, it possesses certain features which differentiate it from the entity usually designated as lymphosarcoma. The cells are perhaps larger and more uniform in size, and an intercellular substance has not been demonstrated. The cells lie in direct apposition, and the cytoplasm of many of them is inclined to be pale or even clear (Fig. 6). The latter feature may, however, be the result of some retrogressive change and may not be observable in cells which are growing vigorously. Comparing this tumor with certain examples of rapidly growing carcinoma, one cannot avoid the thought that, morphologically at least, they have some features in common. Proof of definite relationship is lacking, and from a morphologic standpoint the tumor probably will retain a position between the highly malignant carcinomas and the lymphosarcomas.

The tumor lacks the viciousness one ordinarily would ascribe to other growths which possess many of the same evidences of malignancy, and death seldom ensues as a direct consequence of the

tumor. I have had under observation a dog in the vaginal vault of which such a tumor has persisted for nearly two years; and, while the tumor is slowly growing, the general health of the animal remains good and metastasis cannot be demonstrated. The failure of the tumor seriously to influence the well-being of the animal was a notable feature in the subject of this report. The dog had a normal bodily growth and remained well nourished and playful throughout the period that the tumors were present.

The ease with which this tumor can be successfully transplanted to other dogs, and the manner in which the disease responds to surgical interference, certainly place it in a unique position among the other neoplastic diseases. Considering its unusual clinical behavior, it is not surprising that the cells of the tumor should be found to be somewhat different from other malignant neoplasms. The origin of the type cell is difficult to determine. Although, morphologically, it does have some resemblance to cells such as occasionally are seen in squamous cell carcinomas of extreme malignancy, I am inclined to believe that the cells of this tumor arise from certain undifferentiated lymphocytic forms and that the growth represents a type of lymphoblastoma which can be fittingly designated "transmissible lymphosarcoma." The word "transmissible" is justifiable because of the high percentage of takes secured with this tumor in experiments with transplantation and the striking manner in which it is transmitted to susceptible dogs by sexual contact. These tumors should not be referred to as infectious sarcomas or as infectious lymphosarcomas, since there is no reason to assume that a specific infectious agent is responsible for their causation or that they are transmissible for the same reason. They apparently are transmissible because of the ability of the transplanted or inoculated cells to grow in the tissues of the recipient and not because of the transference of a separate infectious substance in the strict bacteriologic sense.

Summary

The more important literature pertaining to the transmissible lymphosarcoma of dogs is reviewed. A case is described in which two tumors, which morphologically were indistinguishable from transmissible sarcoma of the genitals, appeared respectively in the orbital space and under the skin of the frontal region of a young dog. The genitals were not affected. Although transplantation was not at-

tempted, there seems sufficient histologic evidence to substantiate a diagnosis of transmissible lymphosarcoma. The word "transmissible" is preferable to the older term "infectious," since there is not evidence bacteriologically or otherwise that these growths are dependent for their origin on an infective factor in the accepted sense of the word. It could not be determined whether each growth had a spontaneous origin or whether one represented a metastasis from the other. The possibility of the tumors being the result of transplantations from a vaginal tumor of the mother at the time of birth was considered, but this was not subject to proof since the parentage of the puppy could not be traced.

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DESCRIPTION OF PLATES

PLATE 38

FIG. 1. Appearance of the tumors at the time of operative removal, approximately three months after they were first seen.

FIG. 2. The animal 104 days after removal of the tumors. The wounds had healed completely and recurrence was not apparent.

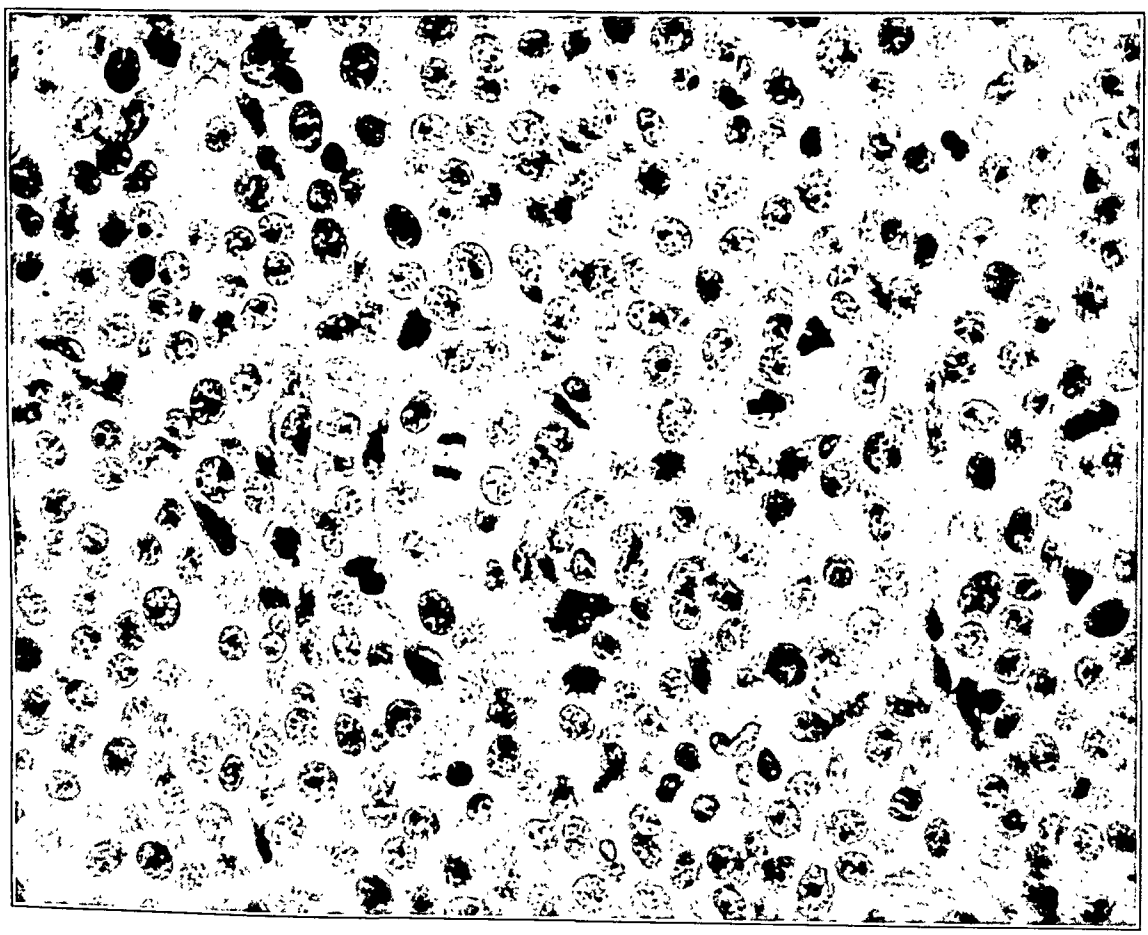
FIG. 3. Section from a typical transmissible or venereal lymphosarcoma from the vagina of a dog. × 660. A similarity may be noted in Figs. 3, 4 and 5.



1



2

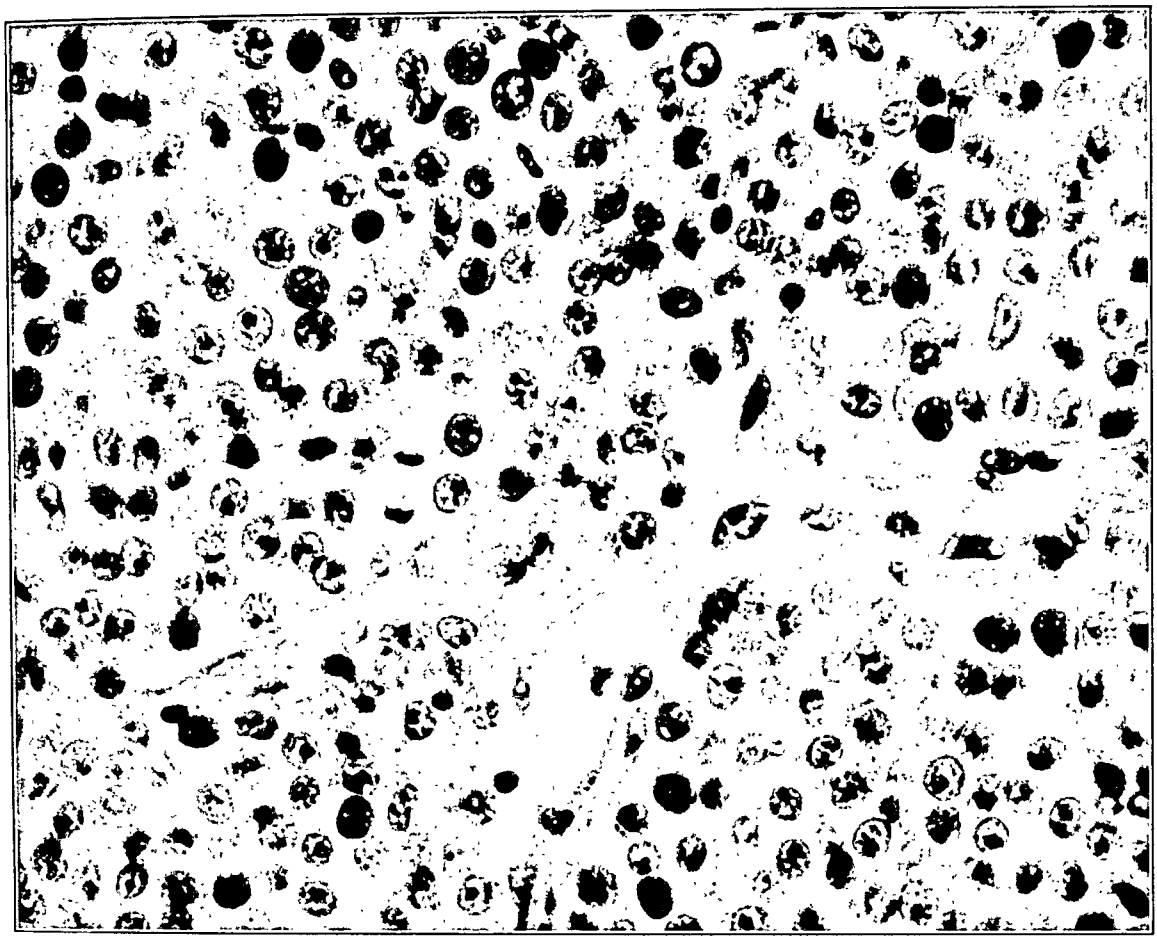


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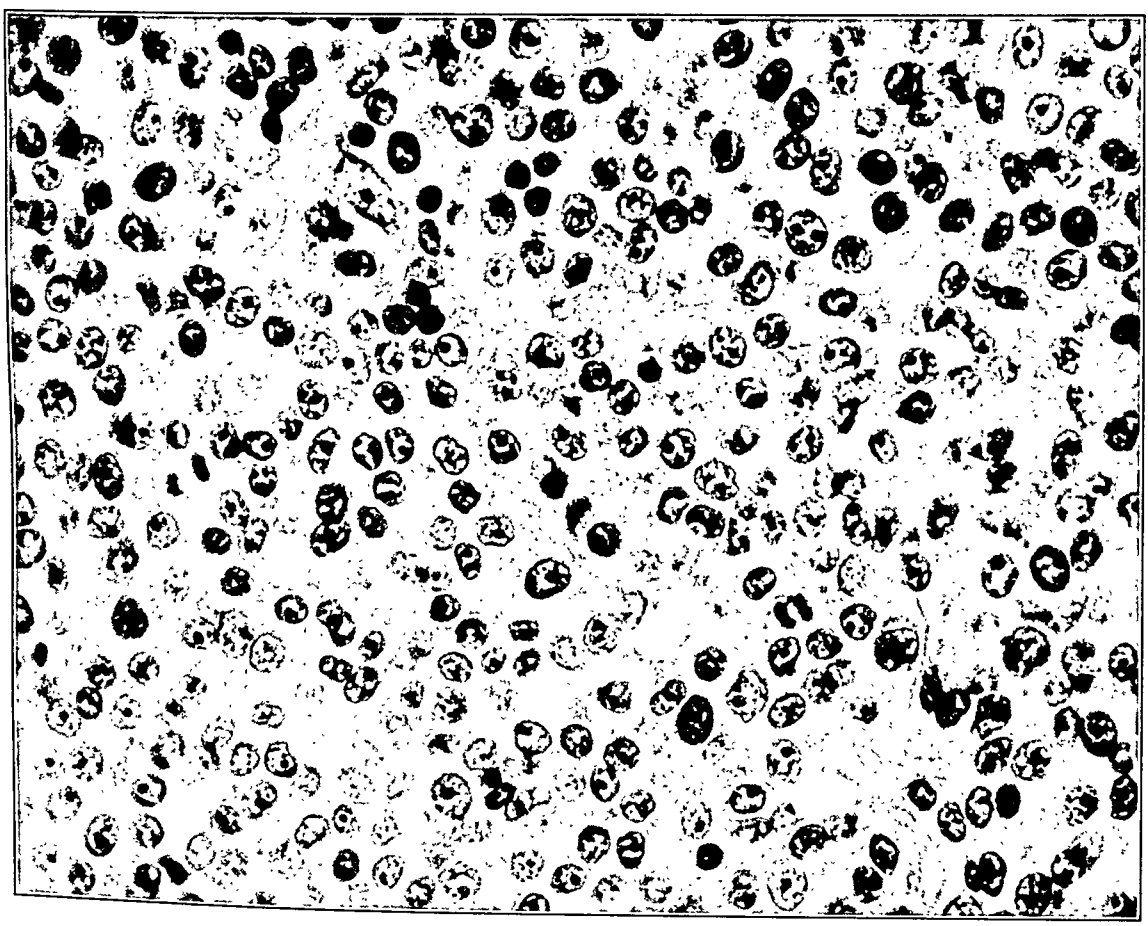
PLATE 39

FIG. 4. Section of the tumor of the eye in the case reported. The close resemblance to the genital tumor in Fig. 3 is apparent. × 660.

FIG. 5. Section of the tumor from the frontal region in the case reported. The structure is similar in every detail to that shown in Figs. 3 and 4. × 660.



4

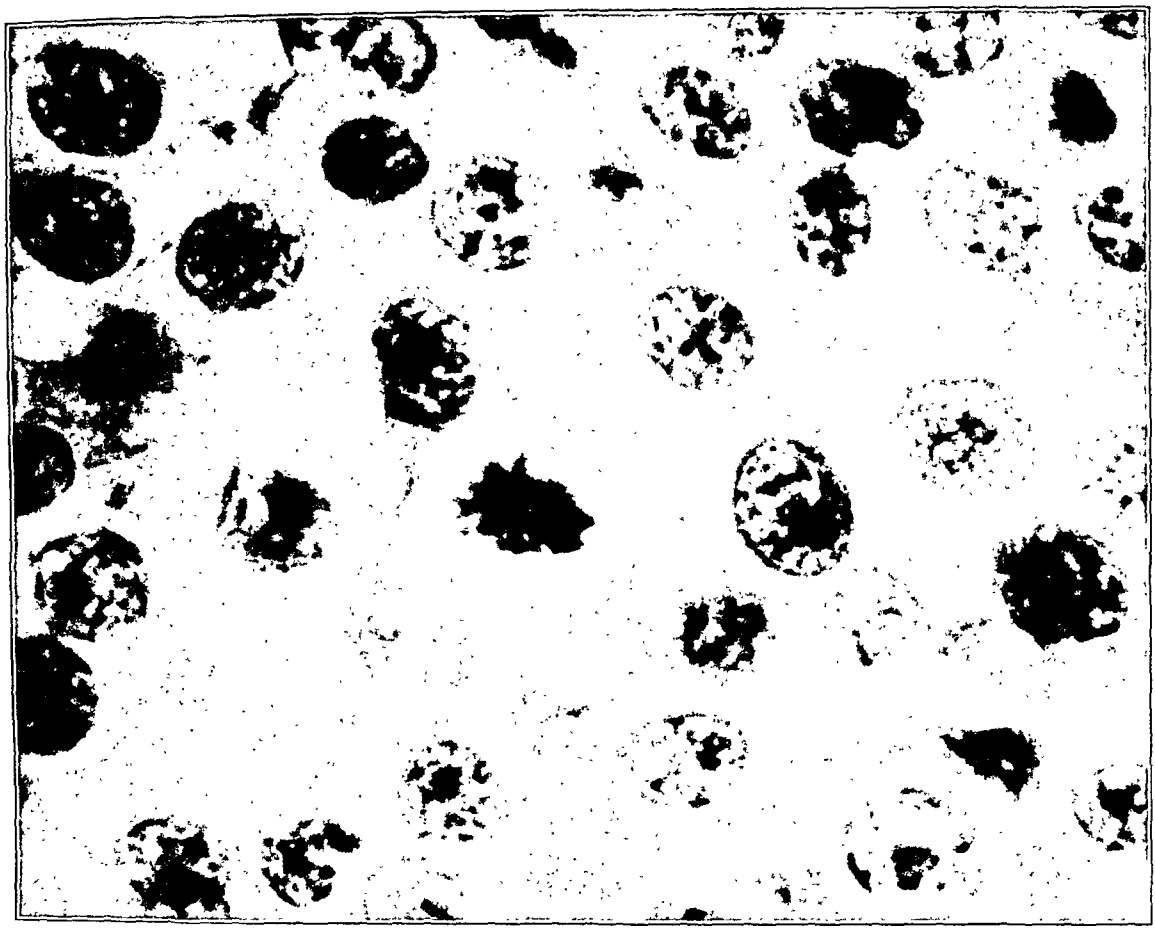


Feldman 5 Infectious Sarcoma of Dog

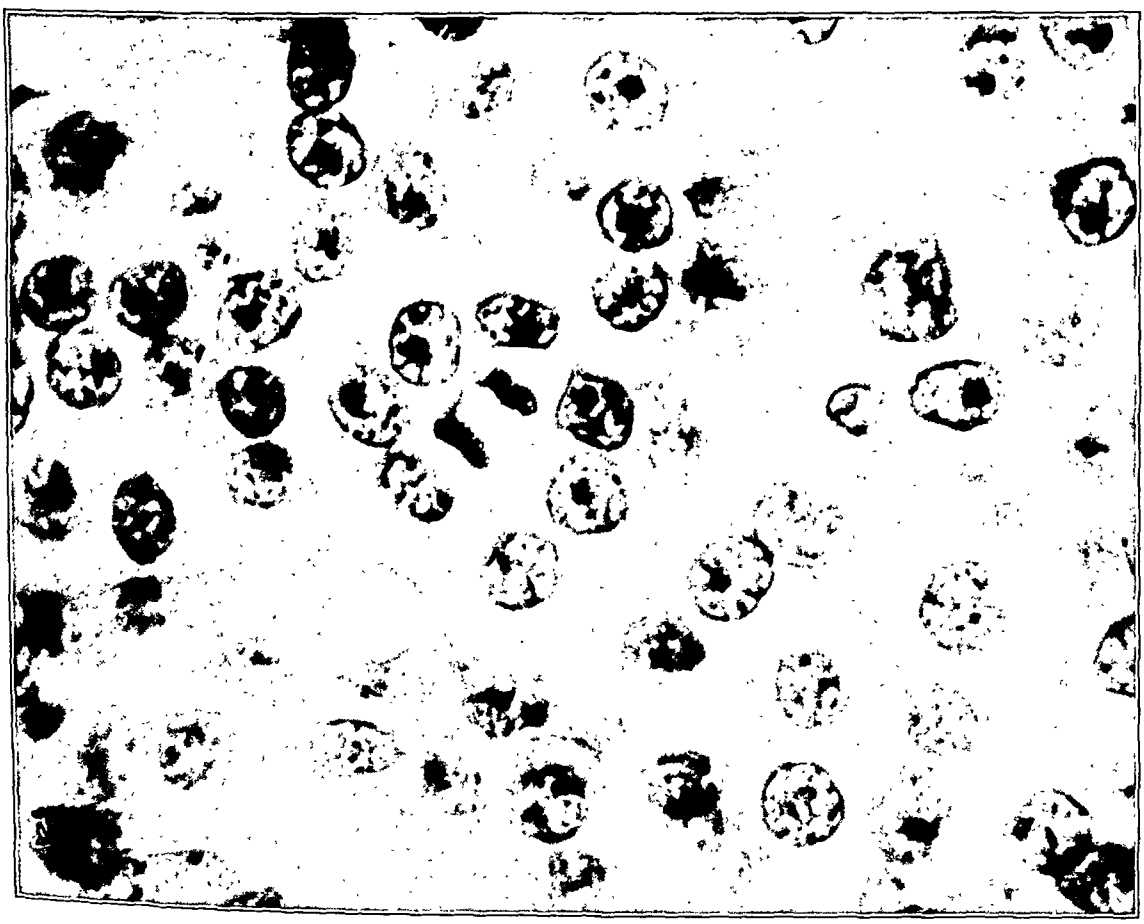
Plate 40

Fig. 6. Section of the tumor of the eye, showing the polygonal outline of the cells and their close apposition. The relative proportion of the nuclear substance to the cytoplasm is shown. One cell is in mitosis. × 2000.

Fig. 7. Section of the tumor of the eye showing the hyperchromatic nuclei and prominent nucleoli. One cell is in mitosis. × 1350.



6



Feldman 7 Infectious Sarcoma of Dog

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MENINGOCOCCUS ENDOCARDITIS *

REPORT OF A CASE

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The number of cases of meningococcus endocarditis reported in the literature is still so small that the meningococcus may be regarded as one of the rarest organisms producing lesions of the endocardium. In more than half of these cases the endocarditis has occurred merely as a complication of meningitis. However, there are rare instances of the meningococcus gaining entrance to the blood stream and involving the endocardium and other structures, leaving the meninges unaffected throughout the course of the illness. The accuracy of the diagnosis of a few of these cases may be questioned because in some the autopsy report is incomplete, and in others there is insufficient bacteriologic evidence.

REVIEW OF LITERATURE

The first case of meningitis in which the meningococcus was isolated from the blood stream was briefly reported by Osler [1] in 1898; and, with regard to the autopsy and bacteriologic findings, was more fully described by Gwyn [2] one year later. The bacteriologic diagnosis was based purely on the morphologic and cultural characteristics of the organism.

We have been able to find but two cases in the literature of endocarditis associated with a meningococcus septicemia in which the existence of meningitis can be definitely excluded. The first of these was reported by Cecil and Soper.[3,4] The patient, a male, 31 years of age, entered the hospital complaining of sore throat, feverishness

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and tenderness of the wrist and elbow of two weeks duration. Physical examination revealed an acute polyarthritis involving the joints of ankles and fingers, and a presystolic and systolic murmur at the apex. The provisional diagnosis was rheumatic fever and endocarditis. A gram negative diplococcus in pure culture was isolated from the blood, but there was no evidence of meningitis and the spinal fluid was normal. Pyrexia increased and the patient became delirious and died on the tenth day of observation. At the autopsy a soft cauliflower vegetation was found on the anterior curtain of the mitral valve. The spleen was large and contained an infarct. The brain, meninges and prostate were grossly and microscopically negative, and smears and cultures from the latter organ showed no gonococci. Gram negative intracellular diplococci were seen in smears and sections from the vegetations. The organism isolated from the blood stream resembled the meningococcus in morphology, cultural characteristics and fermentation reactions, and was feebly agglutinated by antimeningococcus serum.

In the second case, which was reported by Worster-Drought and Kennedy,[5] the clinical course ran over six weeks duration yet no signs of meningitis were present at any stage of the disease. The patient, a male aged 37, entered the hospital with the diagnosis of influenza. The temperature was 104° F yet there were no definite physical signs. After returning to normal for a few days the temperature gradually rose to 105° F. In the second week of observation mitral and aortic murmurs were heard; cyanosis and dyspnoea increased, and in the last weeks, the heart enlarged and the lower extremities became edematous. A gram negative diplococcus isolated from the blood culture in the second week was agglutinated by Gordon's Type II monovalent antimeningococcus serum, but not by the other three types. At autopsy, the brain and meninges were normal. The heart was enlarged, soft and dilated. A fairly tough massive fibrinous vegetation projected into the ventricle from one of the cusps of the aortic valve. The spleen contained infarcts and the kidneys were enlarged.

There are two other cases reported of meningococcus endocarditis without clinical signs of meningitis, one by Warfield and Walker [6] and the other by Rhoads.[7] In neither of these did the autopsy include an examination of the brain, hence the existence of a meningitis cannot be positively excluded.

In the case reported by Warfield and Walker, death occurred five weeks after the onset of symptoms. One week before admission the patient became delirious and developed an herpetic eruption; while under observation there was a high intermittent temperature and a gram negative diplococcus in pure culture was isolated from the blood culture. In the fourth week of the disease a blowing systolic murmur appeared and a few days later the patient died without ever showing signs of meningitis. At autopsy a large gray and red fungating vegetation firmly attached to the mitral valve, projected into the left auricle, while both the spleen and kidneys contained infarcts. The bacteriologic diagnosis of meningococcus was based simply on the morphology and cultural characteristics of the organism.

In the case reported by Rhoads, the patient, a male negro 21 years of age, died in the third week of his illness. Two weeks before admission to the hospital the patient experienced some dyspnoea, palpitation and attacks of precordial pain. Physical examination revealed a diastolic murmur over the aortic area and a loud blowing systolic murmur at the apex. The clinical diagnosis was acute endocarditis with aortic regurgitation. Death followed on the third day after admission without signs of meningitis. At autopsy a smooth, purple, friable vegetation filled a ragged perforation extending through the interventricular septum and involved both the tricuspid and aortic valves. Both kidneys contained infarcts. Smears and sections of the vegetations revealed gram negative diplococci. The diagnosis of meningococcus was based on the morphology of the organism and its colonies, and on agglutination, precipitation, and complement fixation tests.

The four preceding cases are grouped together as having two characteristics in common, first the presence of endocarditis caused by the meningococcus and secondly the absence of any signs or symptoms to suggest a pathologic lesion of the meninges.

In a case reported by Finley and Rhea,[8] meningitis occurred as a late complication. The patient was a male 47 years of age whose illness began three weeks before admission with articular manifestations and attacks of precordial pain with some dyspnoea, cough, chills and sweats. On entrance to the hospital, the heart was dilated and there was a soft systolic murmur at the apex. After being under observation for one week the temperature rose from 101° to 103° F,

the patient became dull and soporose and a hemorrhagic eruption appeared over the abdomen. At this time a positive Kernig sign was elicited, and smears of the spinal fluid showed gram negative intracellular diplococci. A similar organism was recovered from both the spinal fluid and blood. The patient died in the fifth week after the onset of symptoms. The autopsy showed a wide row of slightly pedunculated cauliflower vegetations along the mitral and aortic valves, and a yellow fibrinopurulent exudate at the base of the brain. In this case there is no report of the microscopic findings and the bacteriologic diagnosis of meningococcus is based on the morphology and cultural characteristics alone.

As pointed out earlier, meningococcus endocarditis is found slightly more commonly as a complication of epidemic meningitis, and the following cases are examples of this condition.

Weischselbaum and Ghon [9] reported a case of meningococcus meningitis in a child nine weeks old who died after an illness lasting five weeks. At the autopsy, in addition to the meningitis, fresh vegetations were found on the mitral valve. No details of the bacteriology in this case were given.

Westenhoeffer [10] reported two fatal cases; the first was a child who died after three weeks illness, and the second was a woman, 21 years of age, who lived only five days after the onset of symptoms. Autopsies were performed on both of these, and in each case showed suppurative meningitis and acute endocarditis of the mitral valve.

Markarell,[11] during an epidemic in 1915, had two cases at one time on the same ward. The first was a boy 19 years of age who was admitted with the diagnosis of meningitis. After treatment, this soon cleared up and the spinal fluid became sterile. Subsequently a loud systolic murmur was heard and a blood culture taken at this time showed gram negative diplococci. The condition of the patient gradually grew worse and death occurred twelve weeks after admission. At autopsy a very large friable vegetation was found on the mitral valve, while the brain and meninges were normal. The second case reported by Markarell was a man, 53 years of age, who lived sixteen weeks after the onset of his symptoms and meningococci were obtained from the spinal fluid during his illness. At the autopsy the spinal fluid was turbid and the meninges thickened, but in addition there was a large vegetation on the mitral valve, which almost occluded the orifice. No murmurs had been heard because of em-

physema and consequently the endocarditis had not been diagnosed clinically.

The two remaining cases of endocarditis complicating epidemic meningitis were reported by Fairley and Stewart.[12] A postmortem examination of the first showed an acute meningitis and an acute endocarditis with fresh vegetations on both mitral and aortic valves. Similarly in the second, there was, in addition to the meningitis, an acute endocarditis but in this case the lesion was confined to the mitral valve.

In almost all of these cases of acute endocarditis complicating meningitis the bacteriologic diagnosis of meningococcus endocarditis has been based simply on the morphology of the organisms seen in the spinal fluid. Probably this was sufficient because the identity of an organism is so often revealed by the part of body from which it is isolated, and yet there are exceptions in the literature bearing on this point that are of sufficient interest to warrant mentioning.

Bieck [13] on questionable evidence reported a case of gonococcus meningitis in a patient who had had a chronic urethritis for a year and who died six days after an acute exacerbation of the local condition. Autopsy revealed a patchy meningitis, while examination of the exudate showed numerous gram negative diplococci.

Teacher and Kennedy [14] had a case of micrococcus flavus meningitis following a severe head injury without rupture of the dura; this is of interest because the organism is often regarded as a harmless saprophyte.

Lastly, a case of meningococcus conjunctivitis of a fulminating character in which no general infection occurred, was reported by Ker and Douglas.[15]

Report of Case

Clinical History: D. J. (B. C. H. 572665), a white American housewife, 28 years of age, was referred into the hospital on Oct. 30, 1928 because of malaise, weakness and shortness of breath.

Present Illness: The patient was in moderately good health until three months ago when she had an abscessed tooth extracted. Very soon afterward she began to lose her appetite and vitality and first noticed weakness and shortness of breath which have progressively increased.

About six weeks prior to admission she sustained a transient hemiplegia. Shortly thereafter she gave birth to a child, and within a day or two of this she developed a mild fever, due possibly to a sore throat which she had and which her attending physician, feeling there was no question of puerperal sepsis, con-

sidered an adequate cause. At this time a loud blowing systolic murmur was first noted, and the case was then considered as probably one of endocarditis.

During the six weeks interval preceding admission and following childbirth we have little data. Her appetite diminished, she vomited frequently and lost considerable weight. We are told that she frequently spoke of pains in her finger tips and of numbness and tingling of her hands, also of slight tenderness over her anterior chest wall, and at one time of a single attack of precordial pain.

Her past history is rather vague. We do know, however, that she denied having had tonsillitis, rheumatic fever or chorea; and that her attending physician knew of no time during which she showed signs of meningitis or nephritis.

Physical Examination: A young woman, strikingly pale, moderately dyspneic, poorly nourished and acutely ill, with a temperature of 102° F. Respiration rapid (30), and rather shallow. The skin slightly icteric, warm, soft and dry, with no petechiae. The lips and extremities cyanotic, the hands and feet cold. Sclerae subicteric, mucous membranes pale. Tongue smooth, atrophic and tender. Gums showed some erosion but no bleeding. Few very small pea-sized lymph nodes bilaterally palpable and freely movable in posterior cervical triangles. Thorax not remarkable.

Heart slightly enlarged; apex impulse visible and palpable in fifth intercostal space, 8.5 cm. to left of midsternal line. Greatest diameter of cardiac dullness, 8.5 cm. to left and 2 cm. to right of midsternal line. Sounds are regular but rapid (140) and of poor quality. A loud, rough, rasping systolic murmur and coarse palpable thrill at the apex. The murmur was transmitted to the axilla and over the base of the heart. The pulmonary second sound was accentuated.

The rest of the physical examination revealed no positive findings.

Provisional Diagnosis: Subacute bacterial endocarditis.

Laboratory Findings: Blood showed a marked secondary anemia with slight leucocytosis. Red blood cells 2,200,000, and somewhat achromic. Hemoglobin 34 per cent. Leucocytes 15,500 with 68 per cent polymorphonuclear leucocytes, 9 per cent lymphocytes, 15 per cent large mononuclear phagocytes, many of which were enormously enlarged, irregular, vacuolated and contained both red blood cells and polymorphonuclear leucocytes. The remaining 8 per cent were smudges that could not be identified.

The urine showed a trace of albumen and the sediment contained many white and red blood cells with few casts.

The blood non-protein nitrogen on admission was 100 mg. Two days later it was 195 mg.

The admission blood culture showed gram negative bean-shaped diplococci; a second culture on the following day confirmed this finding.

Progress Note: The second morning the temperature was 103° F, pulse 150 and respiration 40. The patient was irritable, restless and at times irrational.

Discharge Note: No outspoken embolic lesions appeared until the day of death when a few small purplish areas were found on the fingers and toes. The patient became comatose and delirious by turns, screamed that she could not get her breath, became more cyanotic, pulseless and died on the fourth day apparently in uremia.

Final Diagnosis: Uremia, acute nephritis, acute endocarditis, secondary anemia.

Autopsy Report

An autopsy (A28–382), was performed on November 7, five days postmortem. There is edema of the lower extremities. The breasts are slightly enlarged, the areola pigmented and milk is expressed from the nipples. A deeply pigmented line extends from the umbilicus to the symphysis. The vagina is red and ulcerated, and the cervix is unusually soft. No cutaneous petechiae can be distinguished with certainty.

The peritoneal, pleural and pericardial cavities contain 500 cc., 60 cc., and 50 cc. respectively of clear yellow watery fluid.

Heart: Weight 330 gm., slightly enlarged with moderate dilatation on the right side. The myocardium is soft, pale and homogeneous. At about the central portion of the upper surface of each leaflet of the mitral valve, a large, elevated, nodular, yellow vegetation measuring 2 by 1 by 1.3 cm., projects into the cavity of the left atrium. Beneath these fungating vegetations the leaflets are thickened in some places and perforated in others. The adjacent portions of the valve, except for a few delicate pink fibrinous granulations and two thickened and injected chordae tendineae are normal. The remaining valves, the pericardium, endocardium and coronary vessels are normal.

Lungs: They are distended, pale and edematous. The bronchi and bronchioles are filled with watery blood-tinged fluid.

Spleen: Weight 440 gm., is uniformly enlarged and varies in color and consistence. A large, firm, irregular yellow infarct passing through the central portion stands out in sharp contrast to the surrounding soft maroon parenchyma.

Liver: Weight 1740 gm., is pale, moderately firm and slightly enlarged. The fresh surfaces are homogeneous but the finer markings are obscured by a swelling of the parenchyma.

Kidneys: Weight 390 gm., large and firm. The capsules strip readily from smooth pale surfaces. The cortex is regular and 0.7 cm. in thickness. The fresh surfaces bulge slightly and show marked pallor of both cortex and medulla.

Genital Organs: The cervix is congested. Part of the endometrium covering the fundus of the uterus is dark red and covered with a fibrinous exudate. The tubes and ovaries are negative.

Meninges: The inner surface of the dura covering the parietal and frontal lobes is coated with a very thin yellowish glistening rather mucoid exudate. There are three minute petechial hemorrhages in the pia arachnoid covering the cerebrum but there is no visible inflammatory reaction.

Brain and Cord: These were fixed before sectioning and later carefully examined by serial sections. No gross lesions are found throughout the brain, in the ventricles or in the choroid plexus.

Anatomic Diagnoses: Endocarditis; meningitis; infarction of the spleen; ascites; pulmonary edema; endometritis.

Microscopic Report

Heart: The myocardium shows surprisingly little pathology. There are a few small perivascular foci of polymorphonuclear leucocytes; and occasional necrotic muscle fibers are invaded by endothelial leucocytes. The vegetations are composed of a coarse network of fibrin surrounded by clumps of bacteria and covered at the periphery by a layer of polymorphonuclear leucocytes. The bacteria are small gram negative diplococci and many are intracellular. The underlying valve leaflets are necrotic. The pericardium and vessels are normal.

Lungs: The structure of the lung is well preserved. The alveoli contain a little precipitated albumen with scattered foci of fibrin and red blood cells.

Spleen: There is a large area of infarction bordered by an acute inflammatory exudate and extravasated red blood cells.

Kidneys: These show a rather diffuse lesion involving primarily the glomeruli. The capillary tufts are large, cellular and contain little blood. Groups of tubules also show a moderate inflammatory reaction with degenerative changes within the cells and numerous polymorphonuclear leucocytes within their lumina.

Uterus: Slight cystic dilatation of the glands of the cervix, few foci of lymphocytes in the stroma, and an acute inflammatory exudate on the surface of the endometrium.

Tubes and Ovaries: These organs are normal.

Breast: A normal involuting breast, the cells are atrophic and there is little secretion within the glands.

Meninges: The under surface of the dura is covered with a narrow layer of fibrin containing red blood cells and polymorphonuclear leucocytes. A collection of lymphocytes, plasma cells and endothelial leucocytes lies beneath the lining endothelium of the sagittal sinus, and infiltrates the wall of one of the larger vessels of the choroid plexus.

The pia arachnoid is normal except for three small perivascular foci of acute inflammation and hemorrhage. The inflammatory exudate is composed of well preserved polymorphonuclear leucocytes, endothelial leucocytes and little fibrin.

Cerebrum and Cerebellum: Sections from many different parts including the walls of the ventricle and the cervical portion of the cord, show no histologic lesions.

Pituitary: In the anterior lobe there is a small area of necrotic cells that are undergoing phagocytosis.

Microscopic Diagnoses: Endocarditis; meningitis; infarction of spleen; pulmonary edema; subacute intracapillary glomerular nephritis; endometritis. Gram negative bean-shaped diplococci, intra- and extracellular in vegetations on mitral valve.

Bacteriology

An excellent opportunity was offered to study the bacteriology in this case and the results are unusually interesting. A gram negative bean-shaped diplococcus was recovered from the blood cultures taken ante mortem and later from cultures from the vegetations on the mitral valve and from the spleen. Of the smears taken at the time of the autopsy from the base of the brain, from the vagina, cervix and fundus of the uterus and from the vegetations and spleen, only those from the latter two situations revealed a similar organism.

Morphology and Cultural Characteristics: The organism was a small gram negative bean-shaped diplococcus. It varied in size and stained with dilute carbol fuchsin with different degrees of intensity.

In spite of the fact that the organism remained viable in the body for five days postmortem it grew with difficulty under artificial conditions. The most favorable media were glucose ascitic agar and chocolatized sheep blood agar. A scanty growth was obtained on sheep blood agar, Loeffler's serum media, ascitic broth and blood

serum broth, whereas no growth occurred on plain agar or on beef infusion broth.

After twenty-four hours incubation at 37° C the colonies were minute, discrete, moist, round and elevated. They had a grayish blue translucency, produced no hemolysis and formed no pigment. No growth was obtained at room temperature or at 40° C. Cultures placed in the icebox died out in three to four days.

Fermentation Reactions: These were obtained with difficulty using 1 per cent dextrose broth and 1 per cent maltose broth enriched with horse serum. Control tubes were inoculated with known cultures of meningococci and gonococci. No change in reaction was observed in the tube containing the unknown organism after twenty-four hours incubation, while only at the end of six days was the change in the reaction complete. The unknown organism resembled the meningococcus by attacking both sugars and differed from the gonococcus which fermented only the dextrose (Table I).

TABLE I

Fermentation Reactions

Organism	Dextrose	Maltose
Unknown	+	+
Meningococcus	+	+
Gonococcus	+	−
Tube uninoculated	−	−

SEROLOGY

Arkwright [16] pointed out that to identify a gram negative diplococcus accurately one must take into account not only the morphology of the cocci and their colonies, their cultural characteristics and fermentation reactions but also their agglutination and agglutinin absorption reactions with known antimeningococcus sera.

Gordon,[17] in his bacteriologic studies of cerebrospinal fever among the troops, showed that about 98 per cent of all meningococci fall into four groups with minor bonds of relationship between his types I and III, and types II and IV. Furthermore, the monovalent agglutinating sera of types II and IV of the meningococci contain abundant coagglutinins for many strains of gonococci, and different

strains of gonococci vary greatly in their serologic resemblance to meningococci.

Because of this close relationship between these two groups of organisms we performed agglutination tests to determine into which of these groups the unknown organism should fall.

AGGLUTINATION *

Agglutination with Polyvalent Antimeningococcus Serum: Faintly turbid suspensions of twenty-four hour cultures of the unknown organism as well as of four types of meningococci grown on chocolate agar slants were made in salt solution and kept at 56° C for one hour. Four-tenths of a cubic centimeter of bacterial suspensions were added to one-tenth of a cubic centimeter of graded dilutions of standard polyvalent antimeningococcus serum. As controls we used normal horse serum and bacterial suspension, and saline and bacterial suspension. The tubes were stoppered, kept at 55° C for twenty-four hours and placed in the icebox for twenty-four hours before reading.

The agglutination reaction of the unknown organism corresponded very closely to the reactions of the four known types of meningococci. It was complete in a dilution of 1:200 and partial up to 1:800. This, together with the results of the control tests, is shown in Table II.

TABLE II

Agglutination with Antimeningococcus Serum

Strain	Final dilutions Antimeningococcus serum					Saline	Normal horse serum 1:100	Partial agglutination	Complete agglutination
	1:200	1:400	1:800	1:1600	1:3200				
Unknown organism.....	4†	3	2	0	0	0	0	1:800	1:200
Meningococcus Type I.....	4	4	2	0	0	0	0	1:800	1:400
" " II.....	4	3	2	1	0	0	0	1:1600	1:200
" " III.....	4	3	2	0	0	0	0	1:800	1:200
" " IV.....	4	3	2	0	0	0	0	1:800	1:200

† Figures refer to degree of agglutination reaction: 4 = complete.

* The polyvalent antimeningococcus serum, the four known types of meningococci, and the normal horse serum used in these experiments were obtained from the Commonwealth of Massachusetts Antitoxin and Vaccine Laboratory. The types of meningococcus were: Type I, No. 123; Type II, No. 55; Type III, No. 57; Type IV, No. 60, (United States Hygienic Laboratory).

Agglutination with Polyvalent Antigonococcus Serum: Using the same technic as in the former test we replaced the polyvalent antimeningococcus serum with polyvalent antigonococcus serum, and added a known strain of gonococcus as an additional control (Table III).

TABLE III

Agglutination with Polyvalent Antigonococcus Serum

Strain	Final dilutions Antigonococcus serum					Saline	Normal horse serum 1:100	Partial agglutination	Complete agglutination
	1:200	1:400	1:800	1:1600	1:3200				
Unknown organism.....	4	3	2	0	0	0	0	1:800	1:200
Meningococcus Type I.....	4	3	2	0	0	0	0	1:800	1:200
" " II.....	3	2	0	0	0	0	0	1:400	...
" " III.....	2	0	0	0	0	0	0	1:200	...
" " IV.....	0	0	0	0	0	0	0		...
Gonococcus.....	4	4	4	3	1	0	0	1:3200	1:800

In this, agglutination of both the unknown organism and meningococcus Type I was complete in a dilution of 1:200, and partial up to 1:800, which resembled the results of the previous test in which antimeningococcus serum was used. Only partial agglutination resulted with meningococcus Types II and III in dilutions of 1:400 and 1:200 respectively, while the gonococcus agglutination was complete up to 1:800.

The identity of the unknown organism could not be determined from the results of these two agglutination tests alone (Tables II and III), because it was agglutinated equally well by both the antimeningococcus and antigonococcus sera.

AGGLUTININ ABSORPTION

Agglutinin absorption tests were carried out using both polyvalent antimeningococcus and polyvalent antigonococcus sera, a mixed suspension of the four types of meningococci and the unknown organism.

The technic of the first test consisted in absorbing the agglutinins against the four types of meningococci from an homologous polyvalent antimeningococcus serum, and then mixing this absorbed serum with a suspension of the unknown organism. This was accomplished in three steps. First, five-tenths of a cubic centimeter of a mixed

suspension of the four types of meningococci were added to five-tenths of a cubic centimeter of the homologous polyvalent antimeningococcus serum diluted 1:20. After twelve hours at 55° C followed by two hours in the ice chest, agglutination was strongly positive. The mixture was thoroughly centrifuged and eight-tenths of a cubic centimeter of clear supernatant fluid were pipetted off without disturbing the precipitate.

The second step, which was rather a repetition of the first, was carried out simply to insure the complete absorption of the agglutinins against the meningococci. To the supernatant fluid obtained after the first agglutination we added two-tenths of a cubic centimeter of a fairly heavy mixed suspension of the four types of meningococci, giving a final dilution of 1:50. This mixture was kept at 55° C for twelve hours and later placed in the ice chest for two hours. Only very slight agglutination occurred. The suspension was cleared by centrifuging and the supernatant fluid was saved for the agglutination test with the unknown organism.

In the last step, three-tenths of a cubic centimeter of a suspension of the unknown organism were added to one-tenth of a cubic centimeter of the absorbed serum diluted 1:50, giving a final dilution of 1:200. A final reading was made after twelve hours at 55° C followed by twelve hours in the ice chest.

The following protocol (Table IV) shows that the absorption of antibodies from the antimeningococcus serum by a mixture of the

TABLE IV

Absorption Test with Polyvalent Antimeningococcus Serum Absorbed with Mixed Meningococci

Strain	Absorbed serum 1:200	Unabsorbed serum 1:200
Unknown organism	0	4
Mixed meningococci	0	4

four types of meningococci completely removed the agglutinins against the unknown organism. To certify that the agglutinins against the four types of meningococci had been completely removed, a mixed suspension of the meningococci was likewise added to the absorbed serum; and no agglutination occurred. The unabsorbed antimeningococcus serum that was used as a control in this

protocol (Table IV), had been previously subjected to the same treatment as the absorbed serum because the possibility was considered that the prolonged heating, the chilling, and the centrifuging might destroy or alter the agglutinins in this serum.

When the antimeningococcus serum was replaced by antigonococcus serum and precisely the same technic employed, the agglutinins against the unknown organism were again absorbed by the mixed types of meningococci (Table V).

TABLE V

Absorption Test with Polyvalent Antigonococcus Serum Absorbed with Mixed Meningococci

Strain	Absorbed serum 1:200	Unabsorbed serum 1:200
Unknown organism	0	4
Mixed meningococci	0	3

In these two absorption tests (Tables IV and V), absorption of the antibodies from both antimeningococcus and antigonococcus sera was obtained by using a mixed suspension of the four types of meningococci, and in each case the agglutinins against the unknown organism were completely removed as well. This was important for it suggested a close similarity between the agglutinins against the unknown organism and the meningococci.

TABLE VI

Absorption Test with Polyvalent Antimeningococcus Serum Absorbed with Unknown Organism

Strain	Absorbed serum 1:200	Unabsorbed serum 1:200
Unknown organism	0	4
Mixed meningococci	4	4

When the converse of the first agglutinin absorption test was performed, it was found (Table VI) that the polyvalent antimeningococcus serum absorbed with the unknown strain would still agglutinate a mixed suspension of the four types of meningococci. This indicated that by absorbing the agglutinins against the unknown organism, the agglutinins for all the four types of meningococci have

not been removed. If on the other hand polyvalent antimeningococcus serum absorbed with the unknown organism was mixed with the four types of meningococcus separately, agglutination occurred with all but Type I. This suggested that agglutinins for this type had been previously absorbed (Table VII).

TABLE VII

Absorption Test with Polyvalent Antimeningococcus Serum Absorbed with Unknown Organism

Strain	Absorbed serum 1:200	Unabsorbed serum 1:200
Unknown organism	0	4
Meningococcus Type I	0	4
" " II	4	4
" " III	4	4
" " IV	4	4

A final absorption test which suggested even more strongly the identity of the unknown organism entailed incubating polyvalent antimeningococcus serum absorbed by meningococcus Type I with the unknown organism (Table VIII). This table shows that after absorption of the agglutinins by meningococcus Type I from polyvalent antimeningococcus serum no agglutination was obtained from the unknown organism.

TABLE VIII

Absorption Test with Polyvalent Antimeningococcus Serum Absorbed with Meningococcus Type I

Strain	Absorbed serum 1:200	Unabsorbed serum 1:200
Unknown organism	0	4
Meningococcus Type I	0	4

From results of the preceding protocols it would appear that the unknown organism should be grouped as a Type I meningococcus. (U. S. Hygienic Laboratory classification.)

Discussion

Meningococcus endocarditis presents many points of clinical, pathologic and bacteriologic interest. Of the twelve cases which we found in the literature all have ended fatally. In seven of these the cardiac lesions occurred simply as a complication of meningitis and in most cases the endocarditis was not recognized clinically. On the other hand it is important to remember that endocarditis can be caused by the meningococcus without any meningeal involvement and that such cases have been diagnosed as acute rheumatic fever with endocarditis, or subacute bacterial endocarditis. The duration of such cases varies from three weeks to three months. In the case that we have reported in which there were no clinical signs or symptoms to suggest any meningeal involvement, a diagnosis of meningitis was made at the time of the autopsy that was later confirmed by the microscopic examination of the tissue.

Of unusual pathologic interest are the rather typical vegetations — large, firm, localized and fungating, with little tendency to discharge minute emboli. In eight of the cases the mitral valve alone was affected; in three, both the mitral and aortic; in one, the aortic alone, and in one other, the case reported by Rhoads, the interventricular septum was perforated and both the tricuspid and aortic valves were involved.

The bacteriology in these cases of meningococcus endocarditis presents a very interesting problem, and especially in those cases in which there is no associated meningitis. In several of the cases reported, the bacteriologic diagnosis was based merely on the morphology and cultural characteristics of the organisms isolated from blood cultures. These gram negative diplococci, however, form a group of such closely related organisms that such diagnoses are not conclusive. In identifying members of this group the organisms should be agglutinated, and in some cases agglutinin absorption tests performed.

SUMMARY

1. A case of meningococcus endocarditis is reported with the autopsy and bacteriologic findings.

2. A brief discussion deals with the clinical, pathologic and bacteriologic considerations of meningococcus endocarditis.

3. The literature is reviewed.

We are indebted to Dr. F. B. Mallory for the illustration, and to Miss Marion E. Lamb for technical assistance.

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DESCRIPTION OF PLATE

PLATE 41

Photograph of the heart, opened to show the characteristic large fungating vegetations, localized on the leaflets of the mitral valve.



MacMahon and Burkhardt

SUGGESTIONS FOR STAINING TUMORS OF SPONGIOBLASTIC ORIGIN *

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INTRODUCTORY

The work of Globus and Strauss,[1] Bailey, Cushing, and Hiller,[2, 3, 4] and of Penfield [5] and others, based upon that of Ramon y Cajal and his pupils, notably Hortega, has opened a wide avenue of approach to a more intelligent study of tumors of the glioma group; yet pathologists have been slow to avail themselves of the opportunity thus afforded them. There are several reasons for this: the average pathologist is hesitant about forsaking the tried and familiar ground of the hematoxylin-eosin technic for less well known territory; he balks at using the special fixative called for by the Spanish school, and he is disinclined to learn how to interpret the new and unfamiliar pictures obtained in the silver impregnations.

In order to overcome some of these disadvantages, Globus [6] has devised his "new step" for the bromuration of ordinary formalin-fixed tissue, Penfield [7] has adapted this for use in connection with his method for impregnating the oligodendroglia and microglia, and the writer, anxious to make the study of gliomas still easier, has made further modifications in the technic for impregnating sections from these tumors in the hope that the results thus obtained will more closely approximate those with which pathologists are familiar and, at the same time, afford a broader view of the tissues examined and a better opportunity for photographing them.

I have found, while experimenting with the Globus-Hortega method, that the results obtained are open to criticism on three heads: the nuclear detail is apt to be rather meager; the fibers are grayish, rather than jet-black, unless one coat the cells very heavily with silver and further obscure their histologic detail, and the cells that lack argyrophil material are very indistinct; furthermore, the preparations give flat, over-contrasty photomicrographs that are too black-and-white. It was also noted that the use of 1 per cent

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instead of 20 per cent formalin in reducing the silver in Hortega's silver carbonate method, brings out the neuroglia fibers in sharp relief, while it fails to stain the nuclei or cytoplasm of the cells. Instead of being a disadvantage, it will be realized that this is quite the opposite, for the nuclei may then be stained with a dye of another color and the cytoplasm counterstained with one of a third, producing a picture in three colors, instead of varying tones of the same hue.

In order to accomplish this result, the following process has been devised; it is in no way original, being merely a combination of the steps already familiar in the impregnation of reticulum.[8] It consists in utilizing the sharply black impregnation of the neuroglia obtained with a weak formalin reducer and the reliable hematoxylin-Van Gieson technic. It is not yet applicable to paraffin sections, frozen sections must be used invariably.

The Method

Material: Frozen sections of tissue fixed in 10 per cent formalin are cut 15 to 20 microns, thickish sections are more easily manipulated without tearing and the processes of the spongioblasts, which may branch in all directions, are less apt to be cut off in the plane of section.

Bromuration: The sections are washed in distilled water and bromurated by the Globus technique, being placed in 10 per cent ammonium hydroxid for 24 hours, washed briefly in distilled water and transferred to a 10 per cent solution of 40 per cent (concentrated) hydrobromic acid, where they remain for 5 to 24 hours. They are then transferred to Cajal's formalin bromid (3 gms. NH_4Br in 15 per cent neutral formalin, 100 cc.) where they may be stored until wanted. Although the ammonia bath is supposed to serve as a deformalinizing agent, it is found that the sections become more surely impregnated if they have been in formalin-bromid for a few hours. The tissue sections should be handled, throughout the process, with a glass spatula, made by bending about one centimeter of the end of a glass rod to an angle of 60 to 80° and pinching it out thin and flat while still hot and soft, with warmed pliers, so that its broad surface runs transversely, like a scoop. This avoids the tearing of sections so common when glass needles are used, and it facilitates their transferal from bath to bath.

Impregnation: The sections are washed in two changes of distilled water and placed in about 35 cc. of weak silver carbonate solution, which has been heated to about 45° C. The vessel is then set in an incubator at 38° C for half an hour. The silver solution is made up by mixing 50 cc. of 10 per cent $AgNO_3$ with a like amount of 5 per cent Na_2CO_3, shaking, allowing the heavy white precipitate to settle and decanting the supernatant fluid. The precipitate is then washed in 250 cc. of distilled water and again allowed to settle. It is next nearly dissolved, after decanting most of the supernatant fluid, by adding strong ammonium hydroxid drop by drop and continually shaking the graduate, until only a few grayish granules remain undissolved. The solution is then made up to 250 cc. with distilled water and stored in a brown bottle, where it will keep for days. If a precipitate forms in the bottle, it should be filtered off. The used silver diammino carbonate solution, however, must never be poured back into the bottle. The sections become reddish brown in this bath.

Reduction: After washing them in two changes of distilled water, they are brought into a 1 per cent neutral formalin for 2 to 5 minutes; in a few seconds they become ochraceous gray to black and two minutes is usually enough time for complete reduction.

Toning: They are then washed in water, tap-water is no longer inadmissable after reduction is complete, and toned for 2 to 3 minutes in a 1:500 solution of gold chlorid. This keeps well and may be used repeatedly until it no longer removes the yellows and browns from the color scheme of the sections, which indicates that it is exhausted. I use Merck's brown, acid AuCl, the cheaper yellow salt will probably serve just as well.

Fixation: After washing, the sections are fixed in 5 per cent sodium thiosulphate for 2 to 5 minutes, which removes the metallic luster from their surface, takes out superfluous metallic salts and renders the tissue pliant.

Mounting; After washing away the hypo, each section is mounted upon a clean glass slide by being floated over it, one corner pinned to the slide with the glass spatula and the slide removed from the water at a distinctly obtuse angle; bringing it out at an angle too acute with the surface of the water creates eddies that wrinkle the section. Loose or crumpled edges are brought out flat on the glass by immersing the slide edgewise, deeply enough in the water to float

them free, holding it at right angles to the surface and moving it gently up and down until all tabs and loose ends have been straightened out. It is then blotted firmly with several layers of smooth, unpatterned filter paper; timid blotting usually results in the section adhering to the paper, rather than to the slide. If the sections be firmly enough blotted, it will be found that no egg albumen-glycerin adhesive need be used on the slides. The section is then anchored by the Mallory and Wright [9] celloidin method; it is flooded with 95 per cent alcohol from a dropping bottle, then with absolute alcohol and once more blotted dry. A few drops of very thin celloidin are then flowed over the section and gently blown upon until they set in a thin film. The process is then reversed, a few drops of absolute alcohol, followed by 95 per cent alcohol, are dropped over the celloidin and the slide placed in a staining dish of water. The celloidin should be just thick enough to flow in drops and to set in the thinnest possible film that will serve to anchor the sections firmly to the glass. A little experience will teach one how much celloidin should be dissolved in ether, with a little absolute alcohol added to insure complete solution.

Counterstaining: The sections may now be handled in slotted glass staining dishes, like paraffin sections. They are stained for 5 minutes in a fresh solution of Harris' hematoxylin and washed in water until they become blue; warming the water or adding a few drops of ammonia will hasten the bluing. They are then stained for 30 to 45 seconds in Van Gieson's stain (10 cc. 1 per cent aqueous acid fuchsin to 90 cc. saturated aqueous picric acid solution). The stain is poured off as completely as possible and the sections run directly up through 95 per cent and absolute alcohol (omitting a wash in water) into xylol with 5 per cent oil of origanum. After a brief immersion in pure xylol, they are mounted in Canada balsam.

Results of the Method

Nuclei: Brownish red to red, mitotic figures brighter red.

Cytoplasm: Excepting that of the astrocytes, yellowish red to brown.

Astrocytes: The fibrillary type will show black fibrils and a faint red cytoplasm; the protoplasmic type will be almost entirely black, save for the nucleus. "Irritation" astrocytes are gray to black. Vascular processes (sucker feet) are deeply impregnated and black.

Spongioblasts: The cytoplasm is reddish yellow or brownish, the polar filaments black. The apolar variety may show "fibrogenic areas" in black if they be mature enough. Giant cells stain chiefly with the hematoxylin- Van Gieson stain, showing little affinity for silver.

Nerve Cells: These are distinctly brownish, with a few black dendrites and blackish Nissl bodies.

Oligodendroglia and Microglia: Although sometimes well impregnated, these cells are not specifically stained and the method is not yet recommended for their demonstration.

Gitterzellen: Black network on yellowish brown background.

Neuroglia Fibers: Black.

Fibrous Reticulum: Black.

Collagen Fibers: Bright vermilion.

Erythrocytes: Canary yellow to brown.

Granulocytes and Monocytes: These usually show very distinct argyrophil granules.

Areas of hemorrhage and necrosis are very clearly demonstrated and one may thus gauge the presence or absence of degenerative changes very accurately. It is to be noted that the bloated, snow-shoe-shaped spongioblasts and the swollen and vacuolated *Gitterzellen* occur chiefly in the neighborhood of necrotic areas and represent degeneration forms. Pseudorosettes are well brought out, as is demonstrated by Fig. 1.

Advantages of the Method

The advantages claimed for this method are as follows: it gives one a picture in which the nuclei, cytoplasm and neuroglia fibrils are readily distinguishable; the neuroglia and connective tissue are easily identified and kept separate; it affords much more plastic and less chalky photomicrographs than the original technic, and the various stages in the development of the spongioblasts are more readily made out. That the method does not always demonstrate the oligodendroglia and microglia cells in their entirety makes little difference, for tumors arising from these are still imperfectly recognized; Bailey,[10] in his latest paper on gliomas, sets a question-mark after the heading "oligodendroglioma"; if there be microgliomas, they would fall under the heading of sarcoma — as yet none has been authoritatively described.

Hints on the Handling of Frozen Sections

In the first place, it is necessary that the block of tissue be frozen to exactly the proper consistence; this is best done by freezing it through and then thawing out the surface by laying one's finger-tip over it until it feels slight moisture and sections may be cut without their edges splintering and fraying out. It is advisable with some makes of microtome, to "jump" the sections directly from the block into a dish of water that is held in the left hand. With a dry knife, a properly frozen block and a little practice, this is very easy and it eliminates handling the sections before one must. They spread out flat in the water almost immediately. If this cannot be done, one may wet the knife blade and allow ten or fifteen sections to accumulate upon its surface, whence they may be removed in a mass with a moistened finger-tip and floated off this into the water. Removing each section singly with a camel's hair brush is poor practice, for sections are too fragile to be handled in this manner, tearing more readily when they are single than when they are handled in wads of ten to twenty at a time.

Another point to be borne in mind is that it is very inadvisable to try to impregnate too many sections at a time, ten or fifteen, handled separately with the broad, blunt spatula, will be more likely to emerge whole at the end of the procedure than would a large number. Furthermore, they will not tend to stick together and will, therefore, be more evenly impregnated than would a tangled mass. A cheap and easy way to manage the various baths is to provide a number of wide-mouthed 40 cc. phials, which may be stoppered with flat corks and which should be of sufficiently large caliber not to be top-heavy.

Condensed Summary of the Method

1. Frozen sections, cut at 15 to 20 microns from formalin-fixed material are treated for 24 hours each in: 10 per cent NH_4OH, 10 per cent HBr, and Cajal's formalin-bromid, with brief washes in two changes of distilled water between steps.

2. They are impregnated for half an hour at 38° C in a bath of $(Ag\ (NH_3)_2)_2CO_3$ which is initially heated to 45° C.

3. After washing in two changes of distilled water, they are reduced in 1 per cent neutral formalin (0.4 per cent formaldehyd) for two or more minutes.

4. After washing in tap water, they are toned in 1:500 AuCl for the same length of time.

5. After again washing, they are fixed in 5 per cent $Na_2S_2O_3$ (hypo) for a like period.

6. They are washed, mounted on glass slides with celloidin and stained for five minutes in Harris' hematoxylin.

7. They are then "blued" in water, or very dilute NH_4OH and counterstained in Van Gieson's stain for 30 to 45 seconds.

8. Finally, they are run up through ascending percentages of alcohol, avoiding water, into xylol with 5 per cent oil of origanum, pure xylol, and mounted in Canada balsam.

In Conclusion

A method is described whereby the Globus-Hortega technique is combined with the hematoxylin-Van Gieson stain, so that a polychrome picture results. It is believed that this gives slides that are more readily interpreted and have a greater similarity to routine sections than is the case with those impregnated with silver alone. They are also better suited to photomicrography. The writer apologizes for prolixity in this paper, but feels that better results will be obtained if the reader knows exactly how to go about securing them.

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Note: The Spanish literature, covering the work of Cajal and his pupils, will be found listed in the articles of Bailey and of Globus.

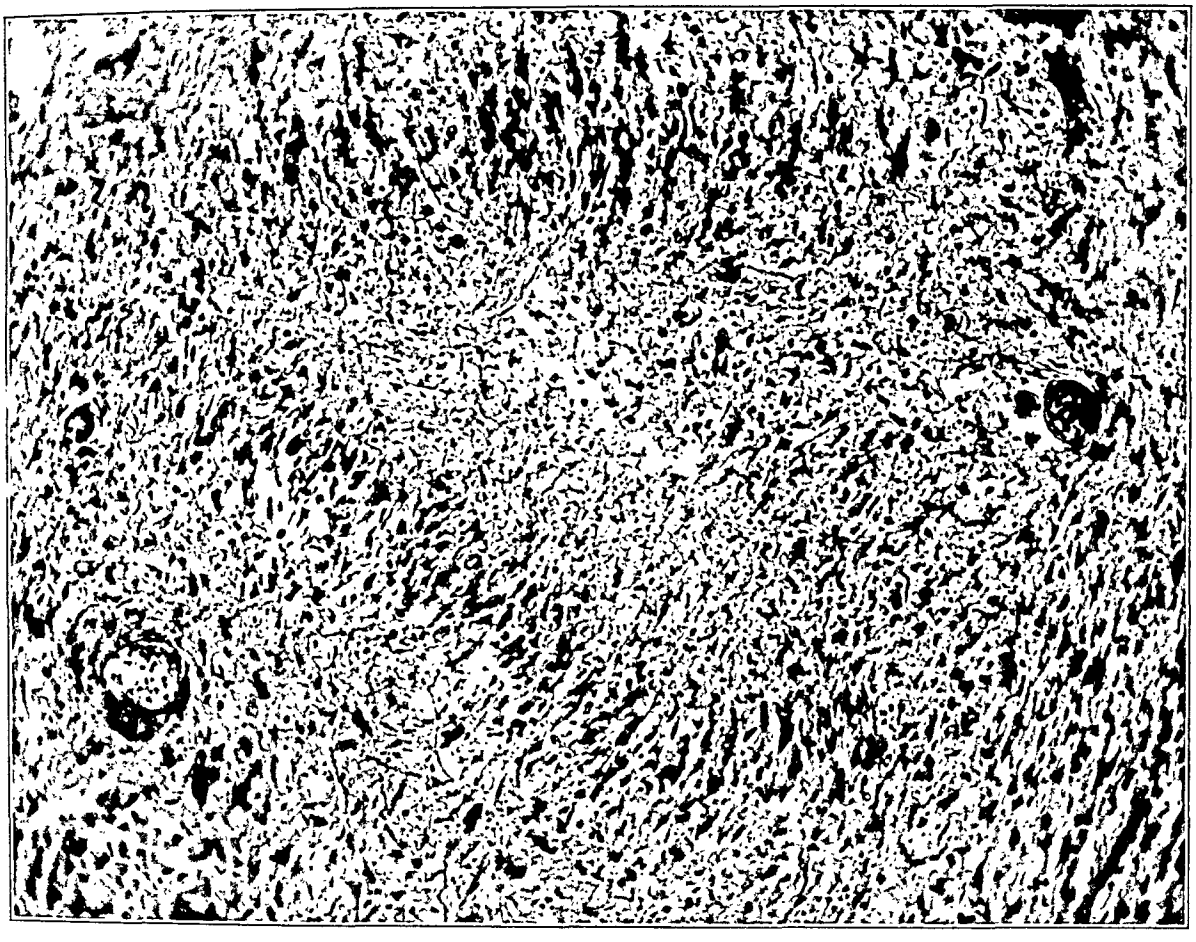
DESCRIPTION OF PLATES

The photomicrographs, taken by Mr. J. B. Homan (of the Department of Medical Art of the College of Medicine) and the author, show a variety of pictures taken from three spongioblastomas. Some fields are selected from approximately normal cerebral tissue in the neighborhood of the tumors. The relatively large magnification of the high power views is the result of extending the photographic bellows to 24 inches in order to secure depth of focus in the thick frozen sections. Everything was done, when printing the photomicrographs, to keep them on the "soft" side and avoid chalky, or over-contrasty effects.

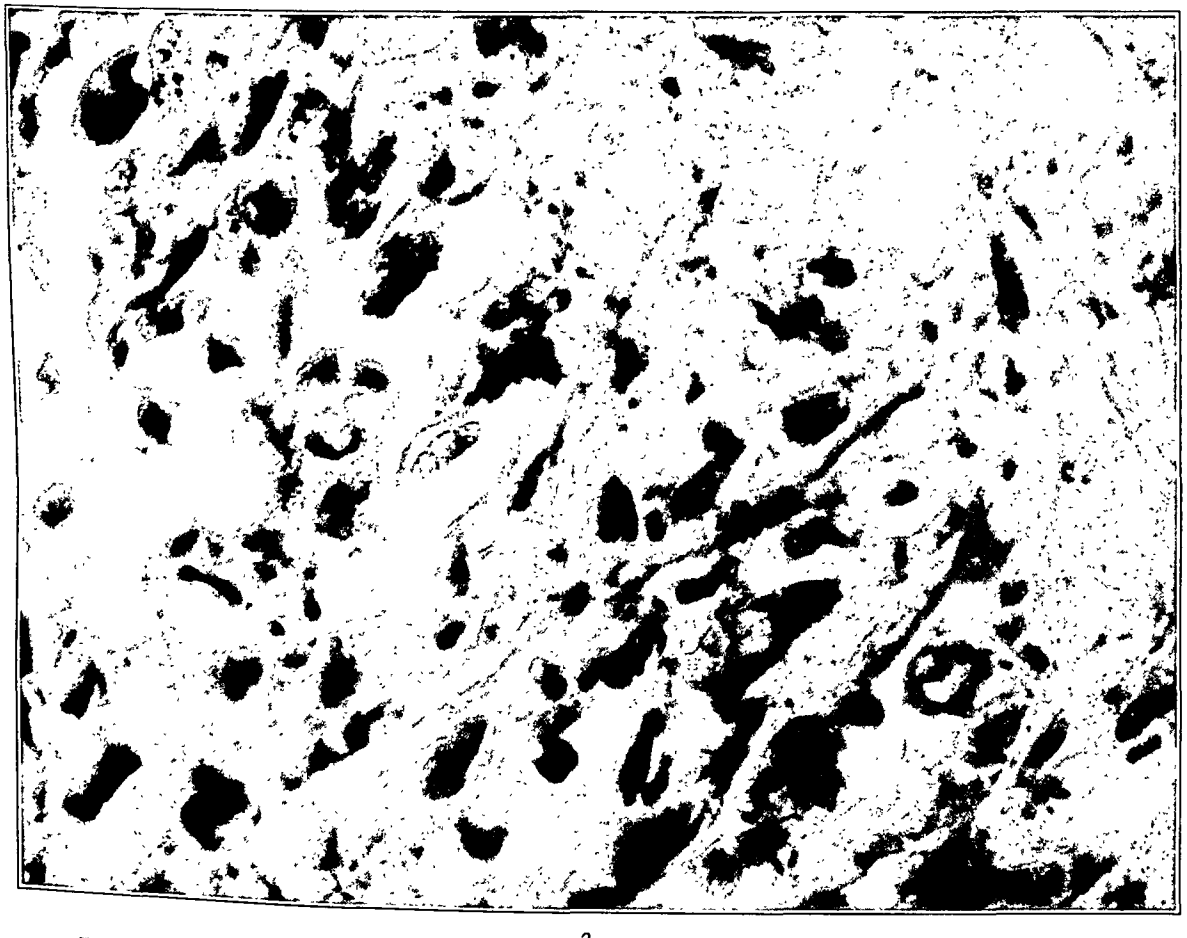
PLATE 42

FIG. 1. A pseudorosette from Tumor 1. Note the polar filaments of the spongioblasts radiating into the necrotic center. × 200.

FIG. 2. High power detail of same. Many giant unipolar spongioblasts are seen, with their "tails" running into the necrotic material. These cells are comparatively poor in argyrophil matter. × 950.



1



2

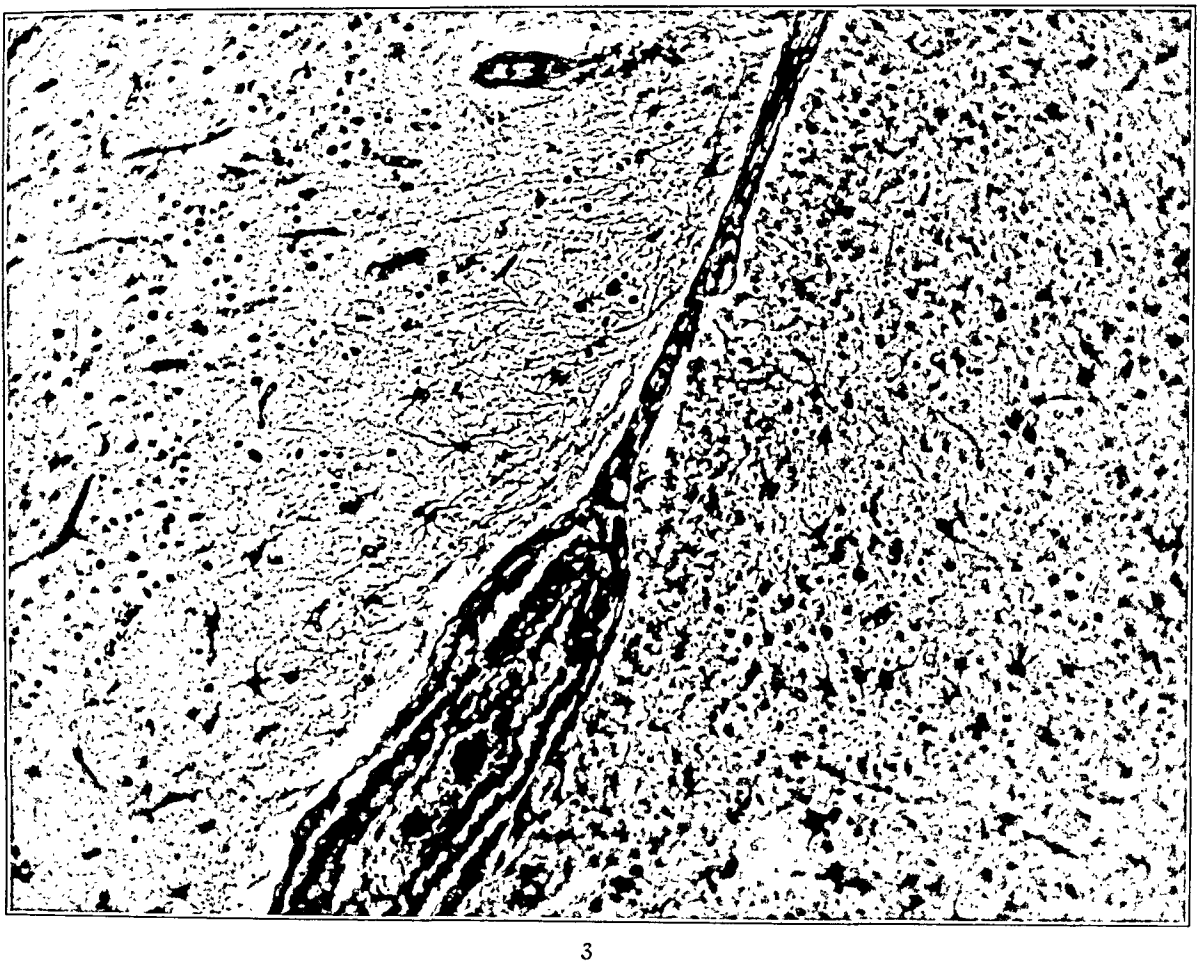
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Staining Tumors of Spongioblastic Origin

Plate 43

Fig. 3. Meningovascular prolongation between sulci, comparatively normal cortex, with fibrillary astrocytes, to the left of the vessel; astroblastic portion of Tumor 1 to the right of it. × 200.

Fig. 4. High power detail from quasi-normal cortex shown in preceding figure. There are two prominent nerve cells, triangular, with scanty processes in the lower corners of the picture; at the center is a cluster of astrocytes; the small nuclei belong to oligodendroglia cells. × 950.



3



4

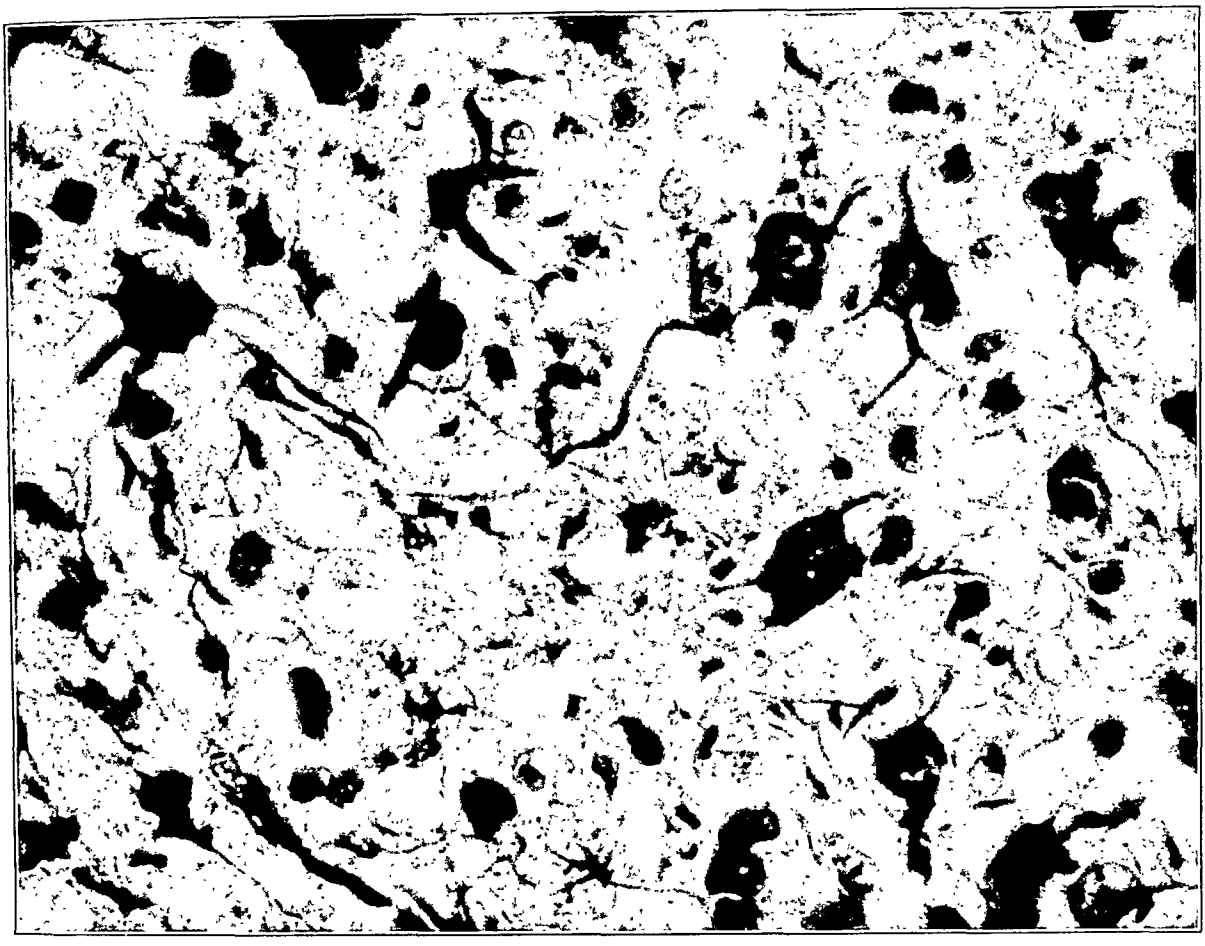
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Staining Tumors of Spongioblastic Origin

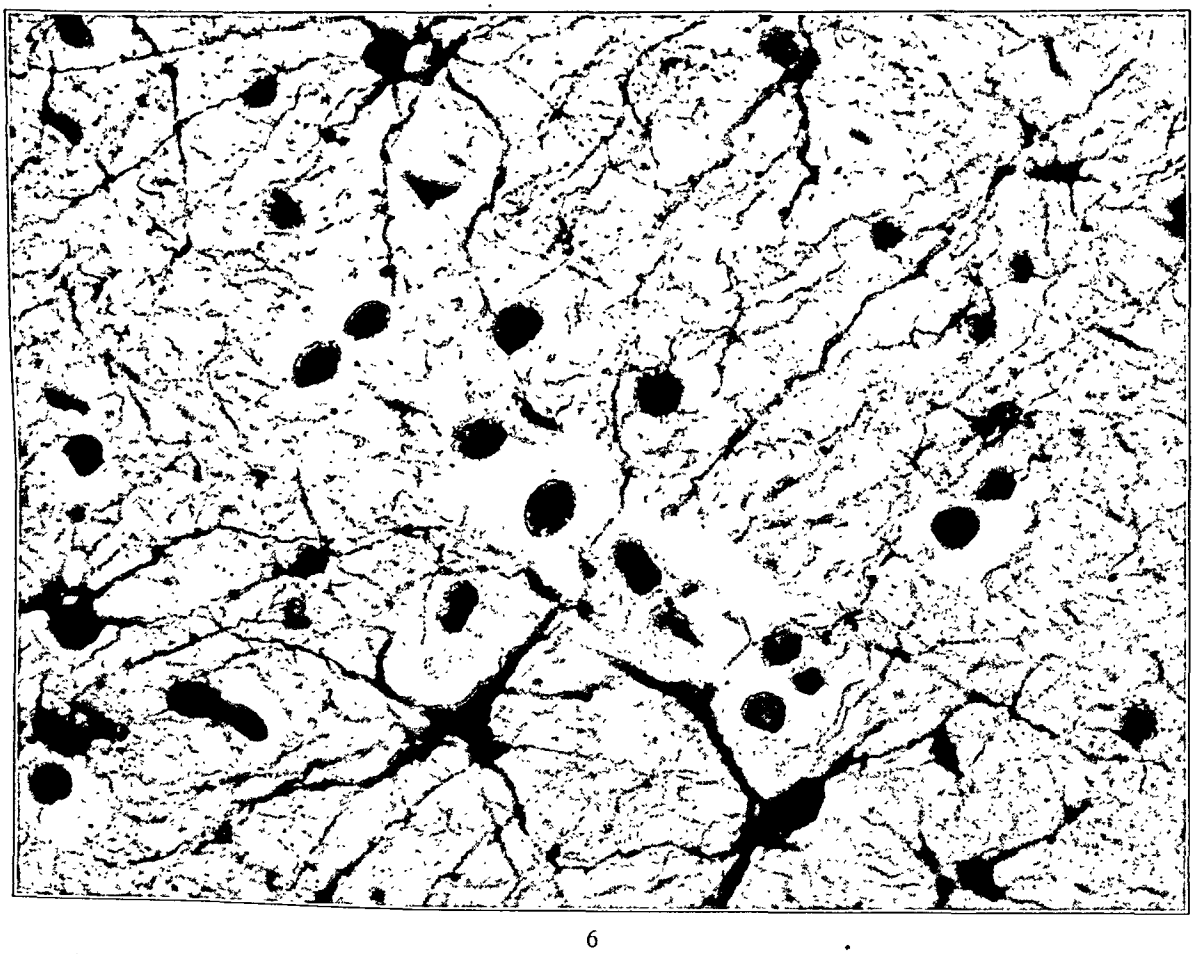
PLATE 44

FIG. 5. High power detail from Tumor 2. Many astroblasts are seen, with rudimentary, thick processes presenting in varying numbers. × 950.

FIG. 6. Fibrillary astrocytes in the white matter near the border of the same tumor. The two at the lower center of the field show very sturdy vascular processes (sucker feet) extending upward to a large capillary which is somewhat out of focus. × 950.



5



6

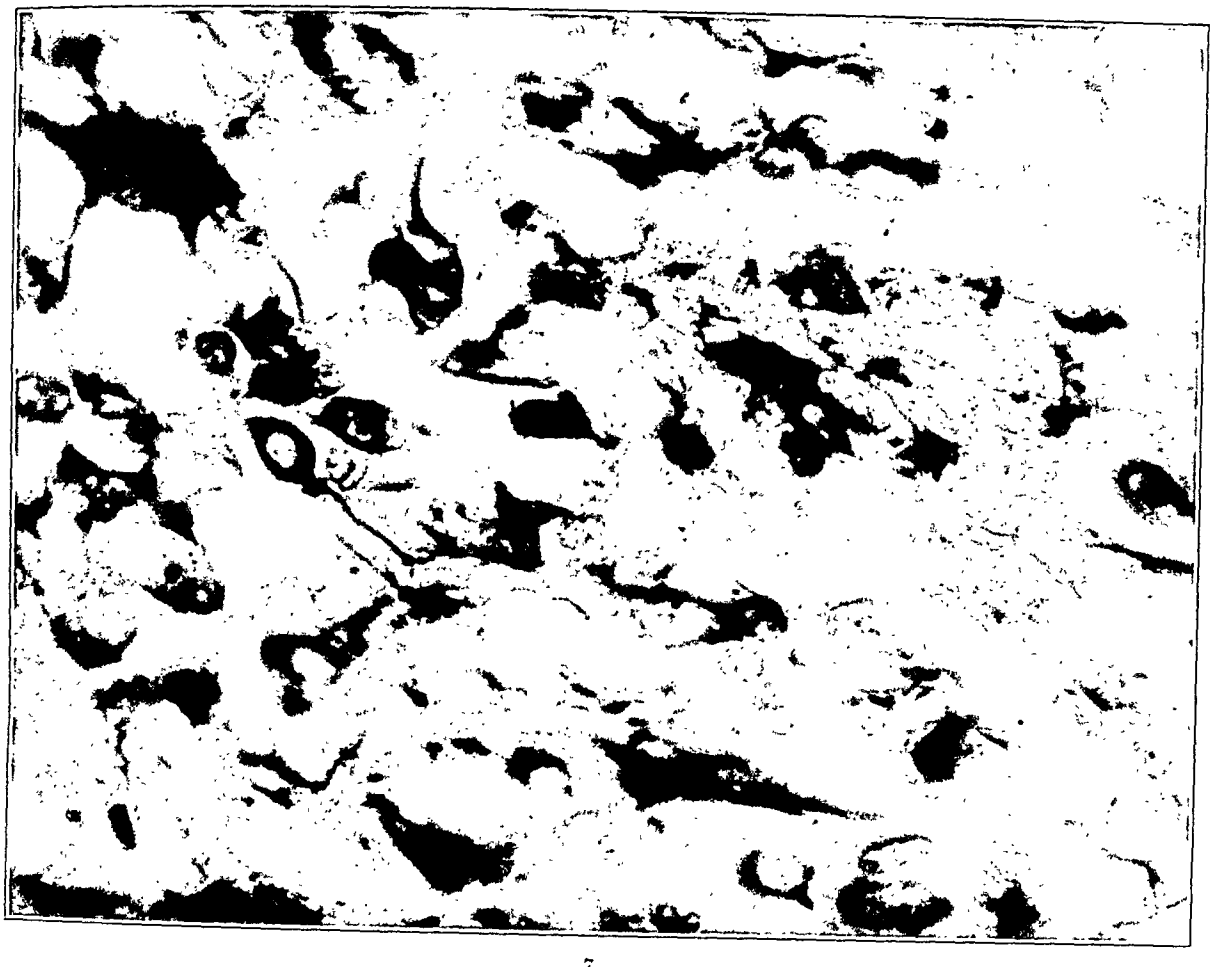
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Staining Tumors of Spongioblastic Origin

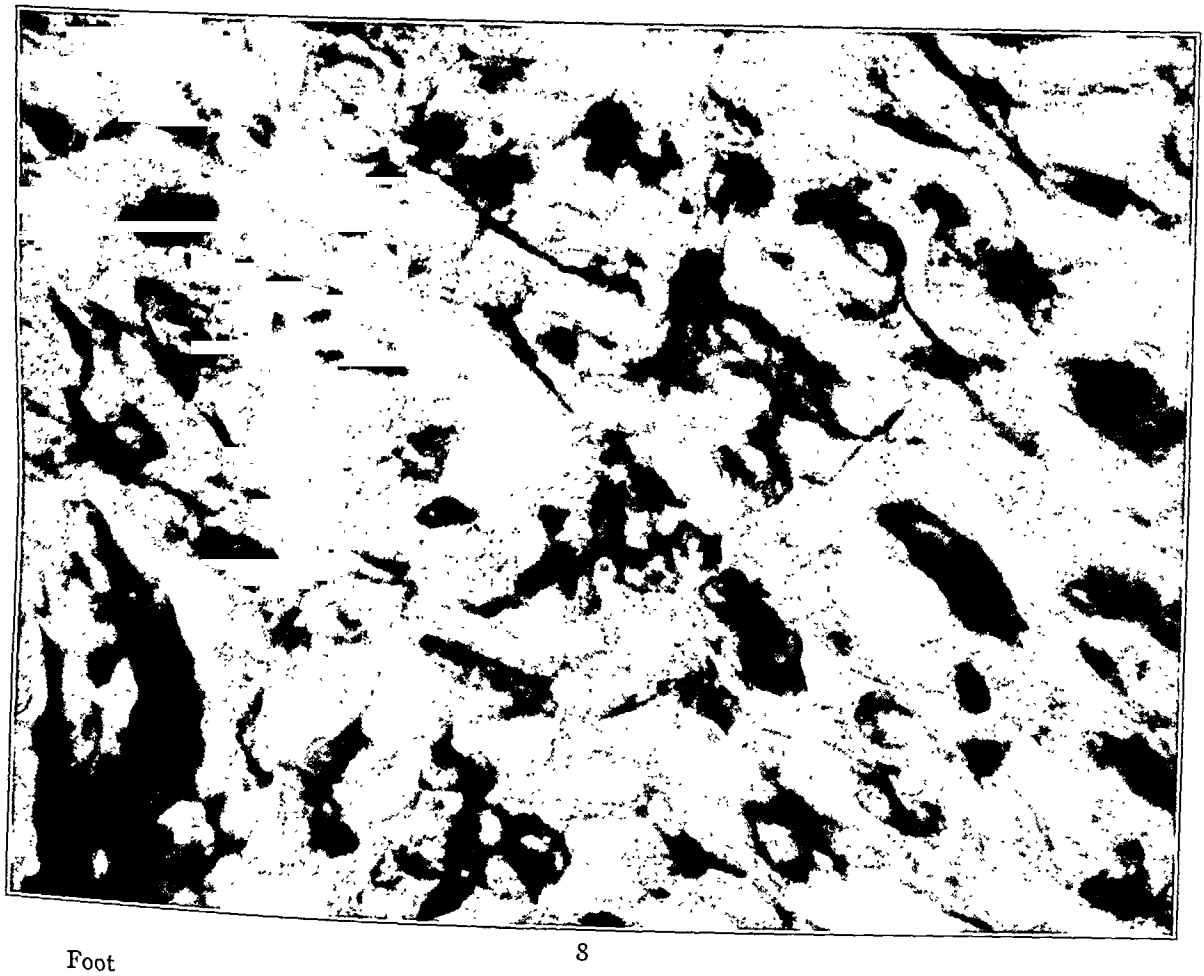
Plate 45

Fig. 7. High power field from Tumor 2. The cells are much more primitive than are those in the preceding tumor, being chiefly of the unipolar variety. × 950.

Fig. 8. Another field from Tumor 2. The cells have much the same characteristics, there are numerous sarcomatoid cells in the background, which show comparatively little argyrophilia. × 950.



7



8

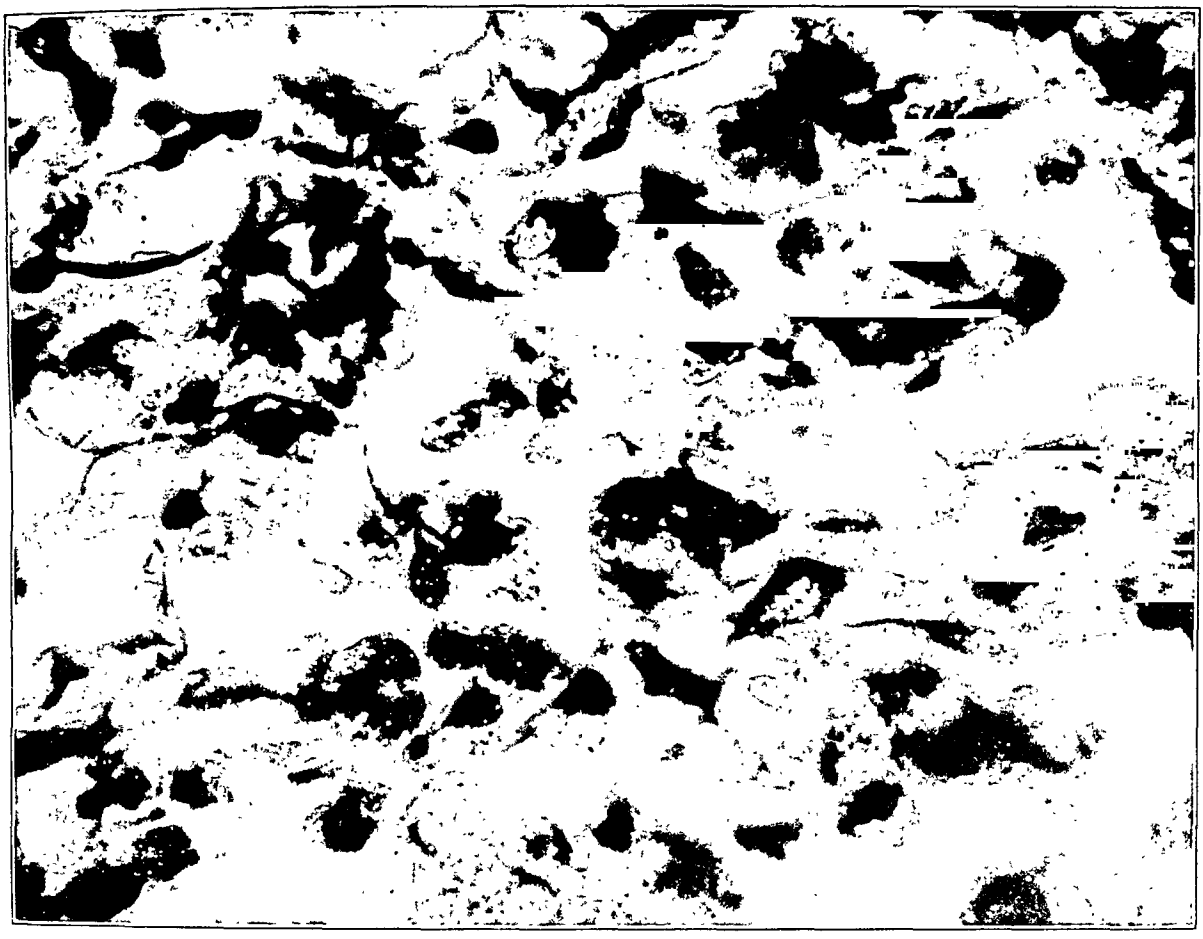
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Staining Tumors of Spongioblastic Origin

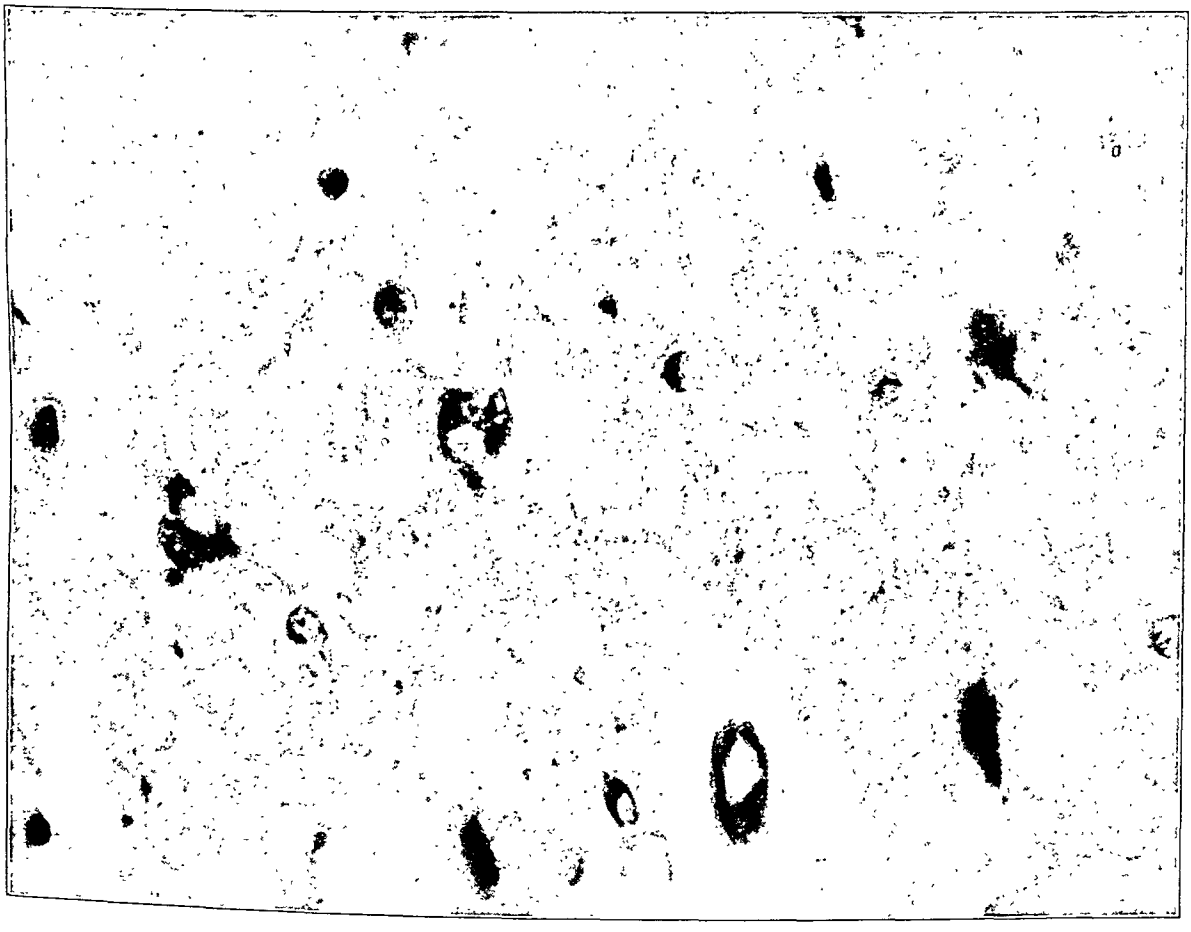
Plate 46

Fig. 9. Degeneration forms, granular or vacuolated and swollen, from the neighborhood of necrotic tissue in Tumor 2. The black fibers are processes of spongioblasts. × 950.

Fig. 10. "Irritation astrocytes" from the white matter bordering Tumor 3. Chiefly of interest because the tissue was kept for eight years in Kaiserling 3 in a museum jar before sectioning and impregnating. × 950.



9



10

Foot

Staining Tumors of Spongioblastic Origin

COMMENTS ON THE IMPREGNATION OF NEUROGLIA WITH AMMONIACAL SILVER SALTS *

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INTRODUCTORY

The purpose of this paper is to present in detail a discussion of the various steps and variable factors involved in the process of impregnating neuroglia with ammoniacal silver salts, so that the reader may obtain some idea as to its regulation and may thus be enabled to alter the procedure to suit his own particular needs. It is probable that few who use silver impregnations know how or why these succeed or fail in producing the desired results. While it will be impossible to answer this question in full, it may be that certain data given here will clear up some confusion and serve as an incentive for the acquisition of more exact knowledge as to the chemical basis of the technic. The method reported in a recent paper (Foot [1]) will serve as the regimen for this set of experiments; although any one of several might do as well, this one is more familiar to me by reason of recent experience.

Material: This was the same in every experiment, cortical tissue from three human brains: one removed one hour postmortem from an elderly man who died of chronic myocarditis and generalized arteriosclerosis; one removed three hours postmortem from an elderly woman dead of septicemia complicated by arteriosclerosis and an area of cerebral softening; and one removed five hours postmortem from a young woman dead of poisoning by bootleg liquor. Thus we have a means for determining the viability of the various neuroglial elements in these brains representing three degrees of freshness. It was found that the astrocytes were viable in all three, but that the oligodendroglia and microglia rapidly deteriorated as the period between death of the tissue and its fixation was lengthened.

* Received for publication April 9, 1929.

Fixation: There is little need to discuss this point; while the Cajal † formalin-bromid fixation is excellent, it is more usual to employ neutral formalin in the pathological laboratory, so that this was invariably used in these experiments in its usual concentration of 10 per cent of the commercial 40 per cent stock, neutralized with calcium carbonate.

After cutting sections at about 20 microns, they were stored in small phials in Cajal's fluid (15 per cent neutral formalin with 3 per cent NH_4Br is the classic formula; it is recommended that the latter be increased to 6 per cent to make it equimolecular with the solutions that follow). A few drops of 1 per cent Na_2CO_3 were added to each phial to maintain the neutrality of the fixative. Here they remained until wanted; after several weeks they were still in a suitable condition for impregnation.

Bromuration: Tissue fixed in neutral formalin must be "bromurated" by the Globus [2] technic before the neuroglia can be demonstrated; it is omitted in those cases where Cajal's fixative has been used. In these experiments the sections were washed in distilled water and placed in 4 per cent NH_4OH at room temperature and at 38° C for 24 hours. Warming brought about no improvement in the ultimate results. They were then washed in distilled water and transferred to 10 per cent HBr at corresponding temperatures for 24 hours. It was found that the best results were obtained by leaving the tissue in HBr in the incubator, rather than at room temperature for 5 to 8 hours. Globus's original technic calls for 10 per cent NH_4OH and it was found to work very well; the percentage was changed for the following reason. The purpose of bromuration is to saturate the tissue with the NH_4Br that was omitted from the fixative; presumably it combines with the silver salts to form AgBr. If this be so, and, if a 0.6 molar solution of $AgNO_3$ be used, we shall need a 0.6 M HBr and a solution of NH_4OH to correspond. Six tenths M HBr is so nearly 10 per cent that this may be retained in the technic, but 0.6 M NH_4OH is 4.04 per cent, so that 4 per cent NH_4OH was substituted for Globus's 10 per cent solution.

The chemistry of bromuration is obscure; it is commonly supposed that the NH_4Br "mordants" the neuroglia for silver salts, but this has never been proved. It certainly suppresses the impreg-

† Throughout this paper "Cajal" and "Hortega" will be used for Ramon y Cajal and Del Río-Hortega.

nation of the neurofibrils and thus affords a lighter background against which the neuroglia is sharply set off when it becomes impregnated; but this is by no means the whole story, for the neuroglia does not become impregnated unless bromuration be employed. That the bromid may, indeed, act as a sort of mordant is indicated by the fact that sections were impregnated after fixation in neutral formalin and the nerve fibers and nerve cells were well demonstrated, without any impregnation of the neuroglia taking place. They were toned with gold, fixed in hypo and washed, then bromurated (the process in no way interfering with the previous or subsequent impregnation), and they were then reimpregnated, the neuroglia now becoming prominent and readily demonstrable. Such double impregnations are very dense and present such a multiplicity of nerve tissue elements that the picture is very confusing; it is, however, none the less instructive and valuable for correlating the position of the various elements.

Cajal's Fluid: The sections were always left in Cajal's fluid overnight, or stored in it until needed, after bromuration. This reinforces the latter and replaces the formalin removed by the ammonia bath, thus insuring good impregnation. Repeated experiments showed that the impregnation obtained after soaking the sections in Cajal's fluid was far superior to that which followed their transfer through a wash of distilled water directly to the silver bath from HBr (Figs. 1 and 2). Deformalinizing the tissue is not to be recommended; apparently the chief effect of the ammonia bath is to react with the HBr to form ammonium bromid in the sections, thus bromurating them as surely as if they had been primarily fixed in Cajal's fluid.

Impregnation: The most complicated and puzzling part of the problem was presented by the various silver solutions used for impregnating the sections. The active ingredient of the silver bath is silver diammino carbonate $(Ag(NH_3)_2)_2CO_3$, or the diammino hydroxid, $Ag(NH_3)_2OH$, as the case may be. The chemistry of these compounds is very well discussed in an excellent paper by Kubie and Davidson [3] and in Mellor's [4] extensive work. The silver diammino carbonate used in the series of Hortega variants is produced by precipitating Ag_2CO_3 from $AgNO_3$ by means of sodium or lithium carbonate and nearly dissolving the precipitate in NH_4OH; the various methods for doing this are conveniently assembled by Pen-

field and Cone [5] in their chapter in McClung's handbook on microscopic technic. It will be noted that only one silver diammino oxid (hydroxid) method is mentioned (Cajal's modification of the Bielschowsky technic), and that the authors believe it to be less certain than the carbonate methods; this will be of importance later on in this article.

In order to analyze these procedures, one must first work out the molecular concentration of the reagents involved therein. In the case of solids, a molar (M) solution would contain an amount equivalent to the molecular weight in grams per liter; thus a molar solution of $AgNO_3$ contains 170 (the molecular weight of the compound) grams per liter, which is 17 gm. per 100 cc., or 17 per cent. In the case of stock solutions, NH_4OH for example, other factors enter in, as will be indicated later.

Silver Nitrate: In all these methods 10 per cent $AgNO_3$ is used, as a 10.2 per cent solution is 0.6 M (102 being 0.6 of 170); this should supplant the usual 10 per cent solution, for it is just as readily compounded.

Lithium Carbonate Methods

The reaction $2\ AgNO_3 + Li_2CO_3 = Ag_2CO_3 + 2\ LiNO_3$, with the $AgNO_3$ in 0.6 M concentration, calls for a 0.3 M Li_2CO_3 solution. Let us see how the familiar formulae are made up.

(*a*) *Original Hortega*: To 10 cc. of 10 per cent $AgNO_3$ an equal, or somewhat greater amount, of a saturated solution of Li_2CO_3 is added.

(*b*) *Later Variant*: To 10 cc. of 10 per cent $AgNO_3$ 40 cc. of the saturated solution of Li_2CO_3 is added. (Saturated at room temperature.)

In either case the precipitate is washed in an excess of distilled water, the wash-water decanted off the precipitate after it has settled and the latter almost entirely dissolved in "dilute" NH_4OH added drop by drop; the solution is then made up to 50 cc. with distilled water.

How do these formulae agree with our theoretical requirements? In the first case there is insufficient Li_2CO_3 to precipitate all of the silver as Ag_2CO_3, but what is precipitated is in pure form; in the second case there is an excess of the lithium salt, which results in

the precipitation of a mixture of Ag_2CO_3 and Ag_2O (see Mellor). Theoretically the two methods should give us quite different results and, as will be seen later, they do in fact.

As was just stated, a 0.3 M solution of Li_2CO_3 is required to balance the 0.6 M $AgNO_3$ in the reaction; working with a 10 per cent $AgNO_3$ and a saturated solution of Li_2CO_3, this is impossible, for the latter is soluble 1.539 $\%^{0°}$–0.728 $\%^{100°}$: at "room temperature" one is uncertain just what the percentage may be, but it is certainly below the figure 2.22 per cent that represents a 0.3 M solution. As 1.5 per cent is approximately 0.2 molar, the $AgNO_3$ solution should be reduced to 0.4 M, or 6.8 per cent, and used in equal amount with the 1.5 per cent Li_2CO_3 to give a balanced reaction. Of the classic formulae, the first gives us pure Ag_2CO_3 but wastes $AgNO_3$, while the second gives us a mixture of Ag_2CO_3 and Ag_2O; therefore they are both theoretically unbalanced. As Na_2CO_3 is readily soluble in water and may easily be adjusted to varying molar concentrations, there appears to be no valid reason why the Li_2CO_3 methods should be retained in use.

Sodium Carbonate Method

Applying the same analysis to this, we find the following to be true.

Ten cc. of 10 per cent $AgNO_3$ is mixed with 40 cc. of 5 per cent Na_2CO_3, the precipitate is washed in a copious amount of distilled water, the wash-water is discarded and the precipitate nearly dissolved in NH_4OH added dropwise. The solution is brought up to 45 cc. and 75 cc. respectively for the "strong" and "weak" concentration. Here there is a great excess of Na_2CO_3; the approximately 0.6 M $AgNO_3$ requires a 0.3 M Na_2CO_3, or an equal quantity of 3.2 per cent Na_2CO_3; what is actually offered to it is four times that amount of a 5 per cent, or nearly 0.5 M (4.68 M), solution. Here again, according to Mellor, we shall be precipitating mixtures of Ag_2CO_3 and Ag_2O.

In order to stabilize the methods, Kubie and Davidson have first produced $Ag(NH_3)_2NO_3$ (silver diammino nitrate) by adding equimolecular NH_4OH solution to a 10.2 per cent $AgNO_3$ until the brownish precipitate that is formed just dissolves. To this they then add calculated amounts of either Na_2CO_3 or NaOH to make their

final double salt and, in the case of the NaOH, add enough more NH_4OH to dissolve the precipitate that comes down when NaOH is added to $Ag(NH_3)_2NO_3$. No additional precipitate will form when Na_2CO_3 is added, as this does not increase the hydroxyl ion concentration, so that no additional NH_4OH is required in that case.

Relative Merits of the Double Salts

It was found well-nigh impossible accurately to judge the relative merits of these methods while using the classic formulae on account of the number of variables introduced by the differences in molecular concentration in the reagents; after a preliminary trial, which used up several gross of slides and a great deal of time and labor, it was decided to employ the Kubie and Davidson methods to decide the questions raised by this preliminary work. It had been found, for example, that sections impregnated with solutions that had been made up with an excess of $AgNO_3$ over Na_2CO_3 were strikingly different from those made up with solutions the reverse of this, with Na_2CO_3 in excess; these differences were attributed to the presence of $Ag(NH_3)_2OH$ [$(Ag(NH_3)_2)_2O$ in solution] in the latter case. This was borne out by the fact that it required twice as much NH_4OH to dissolve the precipitate from the excess carbonate solution as it did that from the excess nitrate solution, although the same amount of $AgNO_3$ was used in both instances, and the precipitate in the former was not twice as copious as that in the latter. This indicates the presence of $Ag(NH_3)_2OH$, which requires more ammonia to dissolve it than does $(Ag(NH_3)_2)_2CO_3$.

Three points were to be tested out: (*a*) the effect of silver diammino carbonate in pure solution; (*b*) that of silver diammino hydroxid in pure solution; and (*c*) that of a mixture of the two. In all instances the reduction of the sections was unorthodox, buffered 1 per cent formalin being used instead of the usual plain neutral 1 per cent formalin. Otherwise the sections were toned, fixed and counterstained in the regular way.

Silver Diammino Carbonate: This was prepared as follows. An 8.1 per cent (1.2 M) solution of NH_4OH was added dropwise to 10 cc. of 10.2 per cent (0.6 M) $AgNO_3$ until the brown precipitate was just dissolved. Then 10 cc. of a 3.1 per cent (0.3 M) solution of Na_2CO_3 was added, without further precipitation occurring. The

solution was then made up to 50 cc. and 100 cc. concentrations with distilled water.

Explanation: The formula of the ion $Ag(NH_3)_2+$ requires a molecular concentration of NH_4OH twice that of $AgNO_3$, or 1.2 M in this instance. Stock NH_4OH has a specific gravity of 0.9 and a percentage content of 28–29 per cent (both noted on the label by the manufacturer). One cc. of this, then, contains $0.28 \times 0.9 = 0.252$ gm., the molecular weight is 17 and a 1.2 M solution would contain 2.04 gm. per 100 cc. Dividing 2.04 by 0.252 we get 8.09 cc. of the stock solution to 100 cc. of distilled water; I used 8.1 per cent as being near enough for practical purposes.

SILVER DIAMMINO HYDROXID: The procedure in this case was the same as that in the preceding; only 10 cc. of 3.1 per cent NaOH replaced the Na_2CO_3 and differed from it in that it produced a reprecipitation that required additional NH_4OH before it became dissolved.

Explanation: The NaOH solution was prepared by making up a 50 per cent solution of NaOH in the hot, allowing it to cool and centrifugating off the pure solution from the impurity of Na_2CO_3 that settles out at this concentration. A 0.6 M solution is needed, as the valence is the same as that of $AgNO_3$. The specific gravity of the 50 per cent solution, as given by Kubie and Davidson, is 1.53; this, times the percentage, equals 0.77 gm. per cc. The molecular weight of NaOH is 40; this, times 0.6 = 2.4 gm. per 100 cc., which, divided by 0.77 = 3.1 per cent.

A third solution was made up according to the Na_2CO_3 technic, but twice the molecular amount of Na_2CO_3 was added in order to afford an excess of Na_2CO_3 over $AgNO_3$. This caused no precipitate. A fourth solution was prepared with an excess of NaOH over $AgNO_3$, to test the effect of this.

Sections were impregnated in all four solutions, on several occasions, with fresh solutions for each test. The bath was warmed to 45° C in a test-tube, poured into a cold phial, and the sections were then put into this and kept in an incubator at 38° C for 30 minutes. The sections in the excess hydroxid bath turned very dark brown, those in the balanced hydroxid bath a deep tobacco brown, those in the balanced carbonate bath a fox red, and those in the carbonate excess bath tobacco brown. Reduction in buffered 1 per cent formalin turned them almost black, olive brown, ochraceous brown, and dark ochraceous brown, respectively. In the gold bath they became black, violet, violet or ultramarine blue, and slate gray, respectively.

The hydroxid sections showed a deep mourning-band of black or dark violet where the cortex lay, a lighter color in the white matter; the carbonate sections were more evenly and uniformly colored.

Under the microscope it was seen that the hydroxid set differed markedly from the carbonate set; in the former the astrocytes were uniformly and beautifully shown wherever they might be, and the nerve cells and their dendrites came out clearly (Figs. 3, 4 and 5), while the oligodendroglia and microglia were not specifically impregnated (see later discussion); in the latter, where pure $(Ag(NH_3)_2)_2CO_3$ was used, the reverse was true (Figs. 6 and 7), the microglia and oligodendroglia being excellently demonstrated, the astrocytes and nerve cells feebly, or not at all. In the excess NaOH set, the impregnation was too dense, tended to incrust the cells with silver and to show troublesome precipitates; in the excess Na_2CO_3 set an intermediate picture was obtained, in which neither group of elements was as well shown as in the case where pure $Ag(NH_3)_2$ + salts were used. With a little juggling in development these intermediate pictures could be improved, but never made as perfect as the others. One gains the impression that $Ag(NH_3)_2OH$ has the greater specificity for astrocytes, while $(Ag(NH_3)_2)_2CO_3$ tends to pick out the oligodendroglia and microglia. The 50 cc. concentration of the latter solution is somewhat superior for that purpose to the 100 cc.

Before leaving the subject of impregnation, it will be well to point out the influence of temperature on the process; it is found that body temperature is better suited to the purpose than greater heat, for hot solutions produce precipitates and incrustations that are very misleading. It has been found that a room temperature solution of the hydroxid will give an impregnation quite similar to that obtained with the warm carbonate. As a rule, a weak bath gives better penetration and more uniform results than a strong one, the impregnation of the microglia being an exception, for these cells come out more strongly and surely if the solutions have been diluted only to 50 cc.

The "equimolecular" method gives much more uniform results, both in regard to certainty and to uniform impregnation throughout the section. One gains the impression that the hydroxid solution is generally superior to the carbonate, giving one greater latitude. It is a mistake to suppose that "spotty" sections are a necessary evil; they are usually a sign that something is wrong. If the reader pre-

fers the classic methods, he should be very careful not to dissolve the precipitate of Ag_2CO_3 or Ag_2O completely; otherwise poor results, or none at all, will follow. It is also always advisable to use doubly distilled water; I redistilled all the water used in these experiments out of a glass apparatus, after it had been distilled through a metal one.

Reduction: Varied effects may be obtained by varying the concentration of neutral formalin used for developing the sections, but as these are fully described by Penfield and Cone, we need not consider them here. In experimenting with the various formalin reducers, ordinary commerical 40 per cent formalin (acid to litmus paper and diluted to 1 per cent), 1 per cent formalin from a 40 per cent solution kept over $CaCO_3$, and 1 per cent formalin slightly acidified with acetic acid were all tested out. The neutral formalin alone gave satisfactory results and is a requisite for successful impregnation.

Kubie and Davidson point out that silver diammino carbonate is not only more stable than silver diammino hydroxid, but it produces better impregnation (when reduced with neutral formalin) than does the hydroxid, owing to the presence of the CO_3 – ion, which buffers the formalin and prevents the formation of formic acid. In reducing silver salts, formalin becomes oxidized to that acid, which is in turn an effective stop to further development, or reduction. Penfield[6] recommends soaking sections in 5 per cent Na_2CO_3 for an hour or more before attempting to impregnate the oligodendroglia and microglia, as he considers that the carbonate acts as a "mordant"; at all events it improves the impregnation. Putting these two facts together, I experimented with buffered solutions of formalin, following impregnation with the silver salts; the results were as satisfactory as they were illuminating.

If 0.5 cc. of 1 per cent Na_2CO_3 be added to 24.5 cc. of 1 per cent neutral formalin, a marked change in the reduction will immediately be evident; the presence of as little as 0.0002 per cent of the carbonate prolongs the reducing action of the formalin, produces a darker reduction and causes the emission of clouds of blackish precipitate from the sections, which should, therefore, be kept in motion. If no carbonate be used, these phenomena are not noted. The intensity of reduction, as observed under the microscope, will be found to have been much increased by buffering the developer.

If 5 cc. of 1 per cent Na_2CO_3 be added to 20 cc. of 1 per cent formalin (a concentration of 0.002 per cent) still greater changes will be seen; the sections are darker, denser, more evenly shaded throughout, and their color remains brown, although slightly intensified and deeper. If equal quantities of Na_2CO_3 and formalin (both 1 per cent) be mixed, or if the percentage of both be doubled so as to make a 1 per cent solution, the sections will become still deeper in general intensity and much redder brown; this percentage of buffer is far too high for good results. In other words, one must get approximately the proper degree of alkalinity in order to obtain the proper degree of reduction.

The changes due to the presence of the buffer are even more marked when the sections are examined microscopically; if from 0.5 to 2.0 cc. 1 per cent Na_2CO_3 (0.0002 per cent — 0.0008 per cent) have been added, it will be found that the microglia and oligodendroglia have been impregnated with $(Ag(NH_3)_2)_2CO_3$ solution, where controls in unbuffered formalin fail to demonstrate them satisfactorily; the fibrils of the astrocytes, in sections impregnated with $Ag(NH_3)_2OH$, will be less heavily incrusted with silver, but much more successfully and continuously demonstrable. Ten to fifteen drops of 1 per cent Na_2CO_3 from a bottle that delivered 1 cc. for each 10 drops, was found to be the most practical addition, and fifteen was the standard for most of these experiments. This is a concentration of 0.0006 per cent of Na_2CO_3; by increasing this to 0.002 per cent, or 5 cc. to 20 cc. of 1 per cent formalin, the following changes were observed microscopically: the background of the section, composed of neurofibrillae and almost suppressed by the bromuration, again became visible although the definition of the fibers was never comparable to that in unbromurated sections (Fig. 8); the neuroglia was less intensely impregnated, its fibers were brownish rather than black when toned in gold and the impregnation approached a stain in its appearance. In sections reduced with unbuffered formalin the fibrillary astrocytes tended to show a pale area where the body should be and the fibers were incrusted with silver; in properly buffered reductions the fibers remained black, but the body of the cell and its protoplasmic processes were brought out as is well shown in Figs. 3 and 5; if over-buffered reducer was used, the cells appeared as though stained with a brown dye and the fibrils were more delicate and less sharply defined. In

properly-buffered sections only, may one expect to impregnate the oligodendroglia and microglia with any degree of uniformity.

Liesegang,[7] in his analysis of the Bielschowsky and Cajal impregnations, states categorically that alkali must not be added to the developer (hydrochinon in that case). This would seem to apply only to the procedure he was investigating and to the impregnation of unbromurated sections, which I have found to be unimproved by reduction with buffered formalin.

To sum up the results of this section on reduction: we may say that the buffering of the formalin developer produces "softer" pictures than the more "contrasty" or harsh results seen after using unbuffered neutral formalin. The reader may try out the method and produce the effect he desires, just as the photographer experiments with "soft," "normal," or "contrasty" developers, plates or papers. One effect of buffering the developer should be stressed, the avoidance of incrustation. If the dendrites and fibrils of a cell come out delicate, clear-cut and continuous from the cell-body to their extremities in buffered solutions, but show heavy, uneven and interrupted outlines (possibly stippled) after buffered reduction, which is more likely to be a normal impregnation and the picture of normal structures, and which an incrustation, or silver deposit, upon such structures? The answer is obvious, and for this reason we must guard against producing artifacts in our desire to get black-and-white sections.

We may assume from what has just been said, that Penfield's "mordant" was, in reality, a buffer that was carried through the silver bath in the interstices of the tissue and acted upon the neutral formalin into which the sections were transferred directly, causing it to retain its reducing power for a longer time. A chemist, reading this paper, may be able to explain why the presence of Na_2CO_3 in the developer tends more and more to bring back the impregnability of the neurofibrillae as its concentration is increased. In some unexplained fashion it offsets the effect of bromuration.

Gold Toning: As Penfield has experimented with the two gold chlorids used for toning (ordinary "yellow" and "brown, acid") it was thought to be unnecessary to repeat his work. The brown, acid chlorid was used, as it happened to be in stock; it is more expensive and, according to Penfield, gives no better results than the yellow, if as good; it is, however, quite satisfactory if the solution be fre-

quently renewed as it pales off in color. When definitely pale, it should be discarded and a fresh golden yellow solution substituted; it takes but a few batches of frozen sections to exhaust the gold chlorid from this very weak (1:500) bath.

As Hortega's "gold sublimate" method gives good results in impregnating astrocytes, it was thought that the addition of 0.5 per cent $HgCl_2$ might improve the toning of their processes and it was therefore used in a few instances; the results were in no way superior to those obtained without the use of 0.5 per cent $HgCl_2$, in fact they tended to be distinctly "muddy" and to produce incrustation of the fibrils. If unbuffered formalin was used for the developer, the mercury did improve the picture somewhat, making it more precise, but the buffered solution is so superior to the unbuffered that this is without significance.

Fixation: No change was made in the usual method of fixing the sections in 5 per cent $Na_2S_2O_3$ (sodium thiosulphate, or "hypo"); it seems to be thoroughly reliable and stabilized.

Counterstaining: In all cases a counterstain with Harris's hematoxylin (5 minutes), followed by a thorough wash in tap water and a second counterstain in Van Gieson's stain, was employed, after attaching the sections to slides by the Mallory and Wright celloidin technic. The silver impregnation tends to give very uneven nuclear pictures, and this tendency is offset by the hematoxylin and nuclear detail rendered uniform, while the Van Gieson stain affords a background that aids ocular inspection and is very useful in photomicrography. The extra 30 seconds entails little loss of time, and the acid fuchsin, by staining the vessels and meninges red, affords valuable landmarks. Liesegang, too, stresses the value of counterstaining with toluidin blue. These counterstains interfere with the silver impregnation in no way, unless they produce unpleasant color combinations or their actinic value be unsuitable for the photographic plate.

Discussion

An extended discussion of the results of these experiments would be beside the point; up to the present we have found out very little of a definitely proved nature about the true explanation of the chemistry of silver impregnation. Liesegang's article and that by Kubie and Davidson stand practically alone in the literature. The

former, it would seem, does very little actual explaining; its author draws an ingenious parallel between brain sections and photographic plates, impregnation being the establishment of "silver-centers" ("Silberkeime") analogous to the "sub-salts" produced by the exposure of a photographic plate to light. Upon these premises he builds up a parallel between the two processes, and one must admit that it appears to be justified; but of proof there is not a shred, the process in the section remains just as empirical as the chemistry of silver salts exposed to light. Liesegang points out that partially reduced silver salts are brown, as the sections become brown in the silver baths they are impregnated with partially reduced silver; development uses the foci of partially reduced silver as centers upon which to deposit more completely reduced metal from the salts that have permeated the tissue. As Kubie and Davidson have shown, the preliminary partial reduction is more prompt and extensive in the case of $Ag(NH_3)_2OH$ than it is in that of $(Ag(NH_3)_2)_2\ CO_3$, which is proved by darker brown sections in the former, redder and lighter brown in the latter instance; furthermore, we know that the former is more unstable than the latter. These authors have performed a number of very interesting experiments to show the difference in behavior of the two. While Liesegang's alluring parallel is very interesting it overlooks the fact that may be of vital importance in distinguishing between the two processes, photographic and histologic; the partially reduced "sub-salts" in the photographic plate, after exposure to light, are quite indistinguishable from the unreduced salts — there is no visible picture until reduction with developer is complete. In the histologic process the partially reduced salts are so visible that the sections that they impregnate might be used for certain purposes of observation; they are essentially sections stained browner in some areas than in others. This point may be totally unimportant, or it may be the reverse.

The experiments described in the body of this paper have adduced a few interesting facts. There appears, for instance, to be a relatively greater affinity for silver diammino hydroxid on the part of the astrocytes and ganglion, or nerve cells, and for the diammino carbonate on that of the oligodendroglia and microglia; whether it depends (as seems likely) upon the difference in the relative instability of the hydroxid as compared with the stability of the carbonate, or upon the difference in the $OH-$ and CO_3- ions, remains

to be seen. Besides this, two more points have been brought out: the superiority of using equimolecular solutions as recommended by Kubie and Davidson and the value of adding a buffer to the formalin developer. If the histologist will follow Kubie and Davidson in making up his solutions, he will be saved a great deal of trouble and avoid many pitfalls; it is not absolutely necessary to use microburets and paraffined bottles, although admittedly safer and more accurate; if the solutions be made up with approximate accuracy and mixed together in trustworthy graduates, the results will be vastly more constant and satisfactory than they will be if one employs the hit-or-miss methods now in use, with now a slight excess here, now a slight deficiency there.

It is just as easy and simple to make up a 10.2 per cent solution of $AgNO_3$ as it is to prepare a 10 per cent solution, just as easy and far more satisfactory to prepare an 8.1 per cent solution of NH_4OH than to drop in a strong solution (percentage unknown if it has stood a while with the dropping-stopper left with its ducts open) or a "dilute solution" of unknown concentration. A glance at my analysis of the Hortega formulae will show how inaccurate one might be in making up a solution according to the Kubie and Davidson formulae and still come much closer to molecular equilibrium. This is not, be it understood, intended as a criticism of Hortega's invaluable contributions to our technical knowledge; it is merely a plea for more accuracy in compounding the reagents used in the methods that he and his colleagues have so brilliantly devised.

The use of buffered formalin for developing impregnated sections is a comparatively insignificant contribution, but it will be found to have its advantages; it permits one to control contrasts in the final picture and renders pretreatment of sections with Na_2CO_3 unnecessary when impregnating oligodendroglia and microglia. It is best to keep down the quantity of Na_2CO_3 used as a buffer to the minimum compatible with good results (overbuffering prevents complete reduction just as underbuffering does); it is probable that someone, reading this, may work out the proper hydrogen ion concentration for the developer. Sometimes, when there is extreme gliosis, or in dense gliomas, it may be desirable to overbuffer the developer in order to bring out detail; the results may be inferior under the microscope, but they are often superior on the photographic plate, using monochromatic light (Wratton B + G filters) of a yellowish green color.

In all this work, frozen sections are essential for success; the use of alcohol and chloroform in embedding in paraffin appears to preclude successful impregnation. Liesegang mentions that alcohol, and ether to a greater extent, prevents the impregnation of brain tissue with silver salts; I noted the same fact independently last year, in connection with the impregnation of the connective tissue reticulum (Foot [8]). The alcohol seems to change the potential of the tissue, by displacing the formalin, for frozen sections fixed or treated with alcohol and found to be unimpregnable became readily impregnated after they had been refixed in formalin. Liesegang gives a similar explanation. Whether we shall be able, after embedding and sectioning material in paraffin, to bring back the power of the tissue to reduce silver partially and permit successful impregnation, remains to be tried out experimentally; the removal of most of the lipins from cerebral tissue by the extraction that is a concomitant of paraffin embedding may prove to be the chief obstacle. Although many, myself among them, have already attempted to impregnate paraffin sections of brain, the results have thus far been worse than mediocre.

Conclusions

1. The technic recommended by Kubie and Davidson, of the Rockefeller Institute, for preparing solutions of ammoniacal silver salts for the impregnation of brain tissue has been found, on trial, to give results commensurate with its chemically correct basis and far superior to those obtained by using the more roughly prepared solutions now in general vogue.

2. It is found that silver diammino hydroxid gives beautiful results in the impregnation of astrocytes, while silver diammino carbonate concentrates chiefly upon the oligodendroglia and microglia. If mixtures of both salts are used, as is the case in most of the inaccurate methods now employed, the impregnation is of a more general nature, but inferior in its specificity to that obtained through the use of pure salts.

3. If the 1 per cent neutral formalin in general use for developing sections impregnated with either of these double salts be buffered with a little sodium carbonate, a definite improvement in the development of the sections follows; the impregnation is more uniform, freer from incrustations and precipitates and shows more precise definition of the neuroglia.

4. No one method is recommended, the reader being left to draw his own conclusions and choose for himself that technic which will suit his particular needs; it is considered that the subject is fully enough set forth to afford him an opportunity for devising his own procedure. The excellence of silver diammino hydroxid should, however, be stressed.

NOTE: All the photomicrographs were taken by Mr. J. B. Homan, of the Department of Medical Art, with the collaboration of the author. They represent a magnification of × 800 diameters. In every instance the sections were counterstained with Harris's hematoxylin and Van Gieson's stain.

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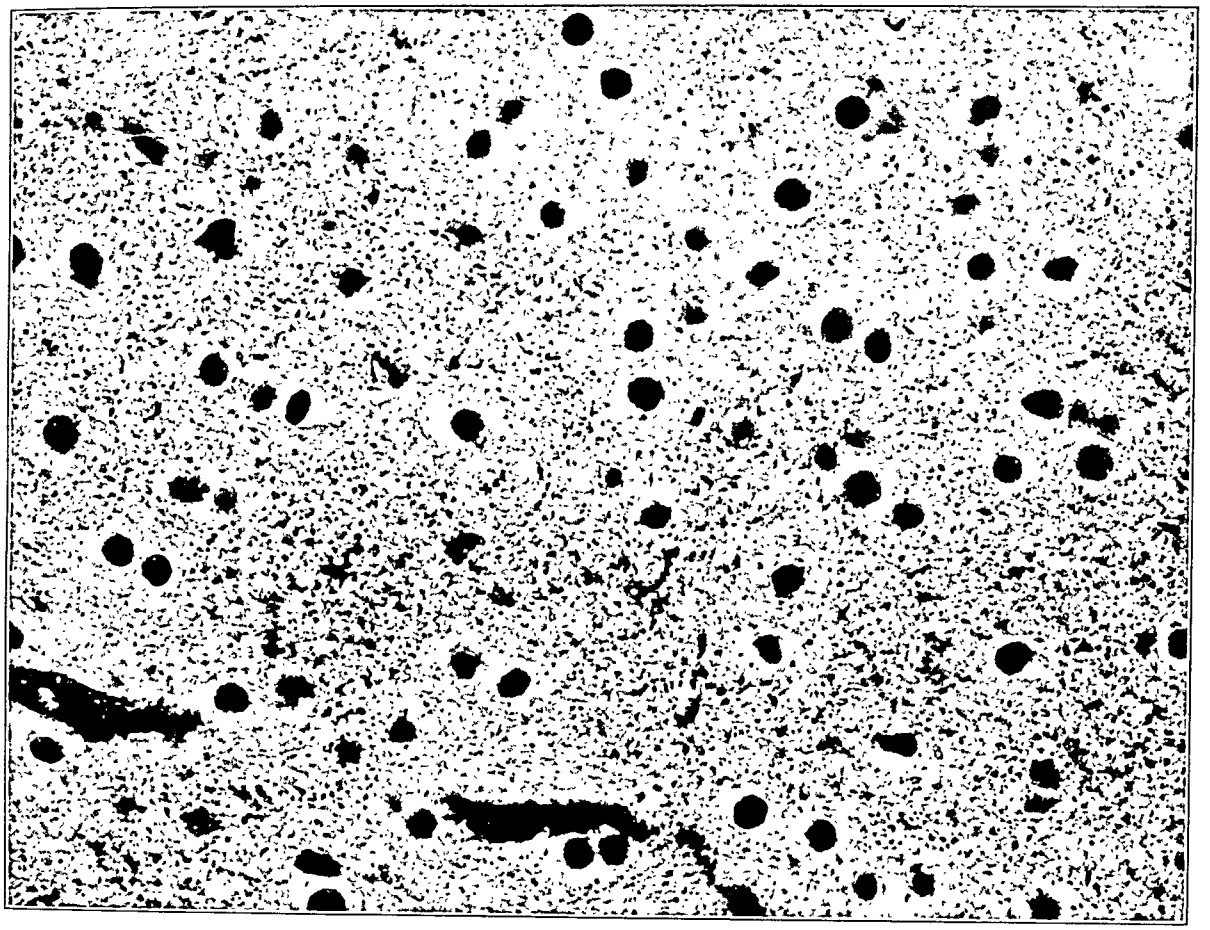
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DESCRIPTION OF PLATES

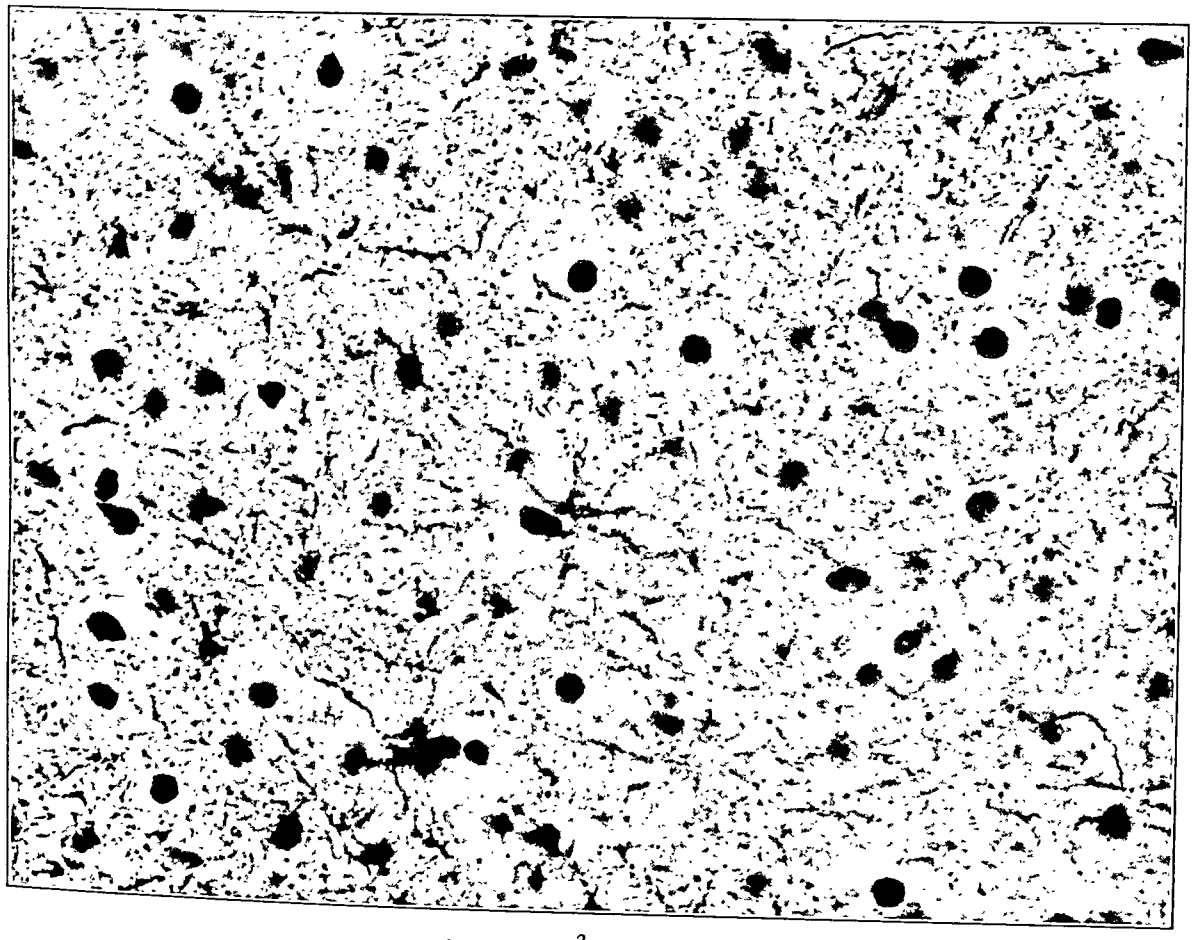
PLATE 47

FIG. 1. A field that should show astrocytes, impregnated with ammoniacal silver carbonate made up according to Hortega's first formula with lithium carbonate, after direct transfer of the sections from a wash in distilled water following hydrobromic acid, omitting Cajal's fixative.

FIG. 2. The same type of field, from the same case (3), following the same technic but interpolating an overnight soaking in Cajal's fixative. The astrocytes and their fibrils become apparent, although not particularly well impregnated in this instance.



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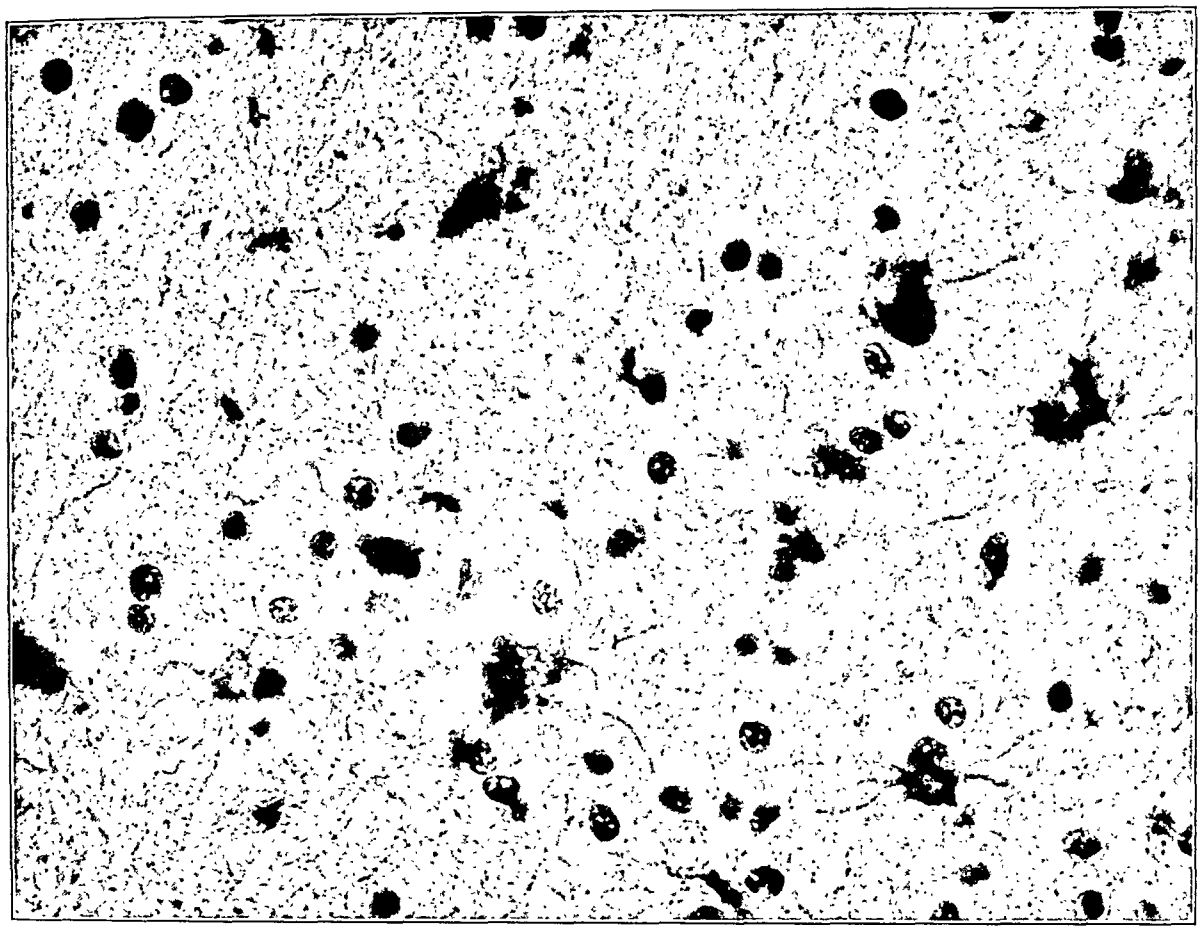
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Impregnation of Neuroglia

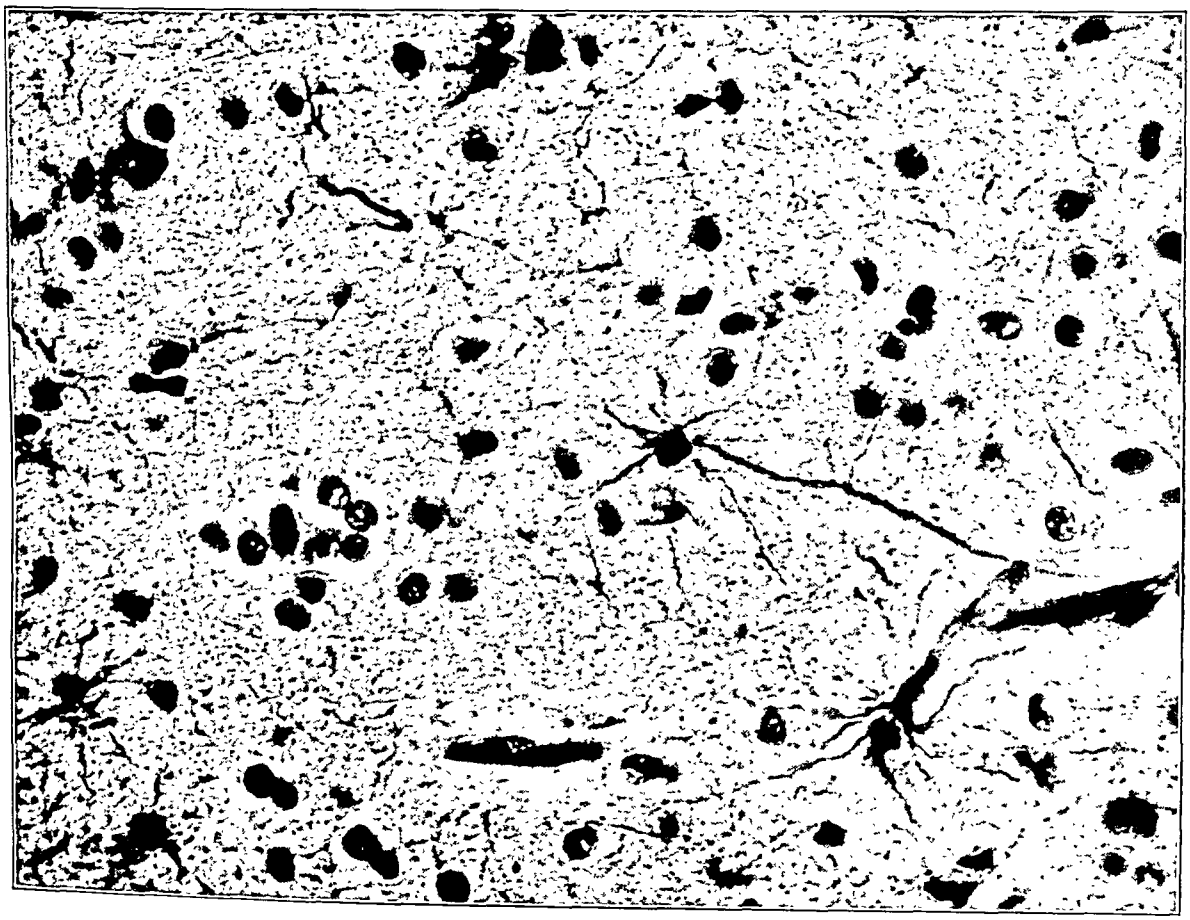
Plate 48

Fig. 3. Large-bodied astrocytes, resembling "irritation forms" seen in gliosis, impregnated with ammoniacal silver hydroxid. White matter of cerebral cortex in Case 1.

Fig. 4. Fibrillary astrocytes impregnated by "Hortega weak ammoniacal silver carbonate" method and developed with buffered formalin. The fairly good impregnation presumably depends upon the presence of silver diammino hydroxid, due to excess sodium carbonate. Case 3.



3



4

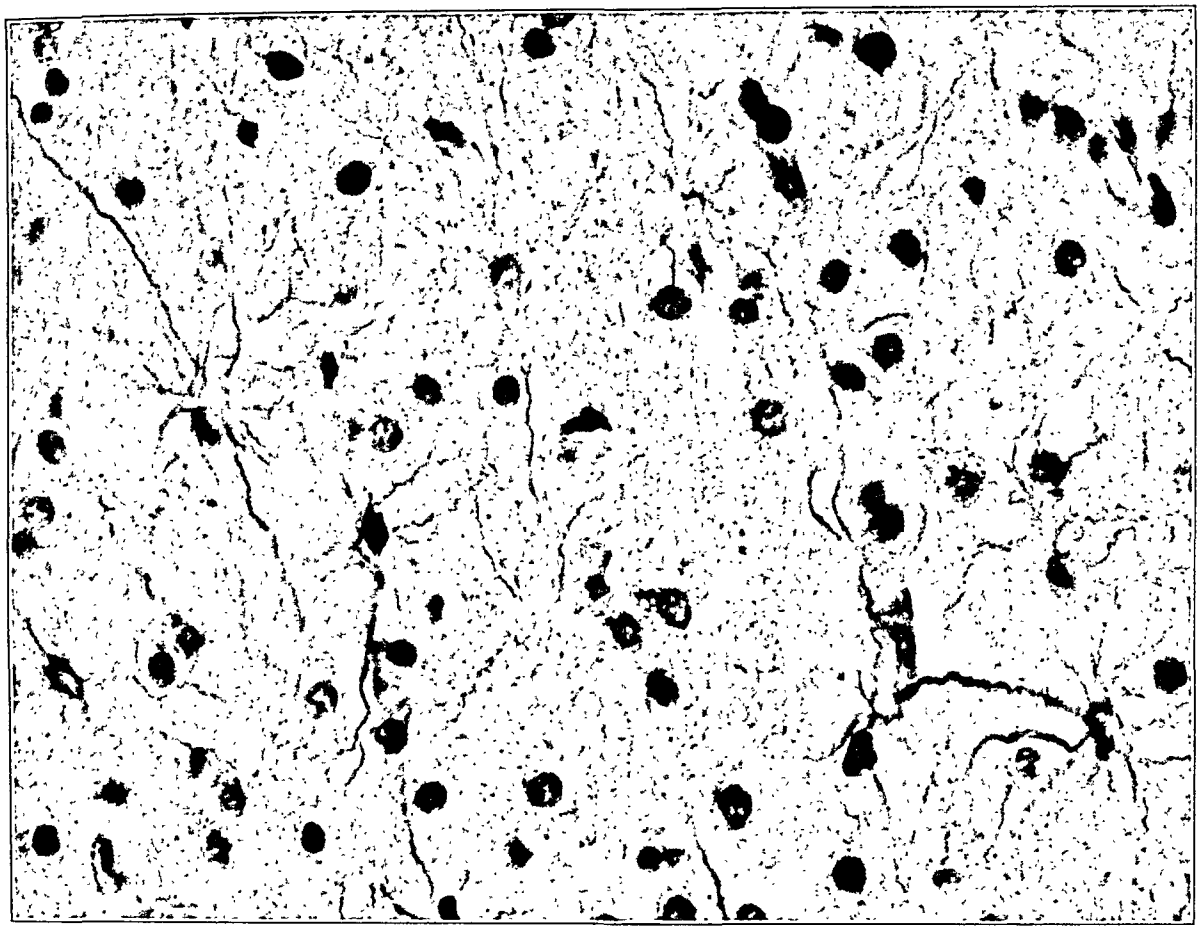
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Impregnation of Neuroglia

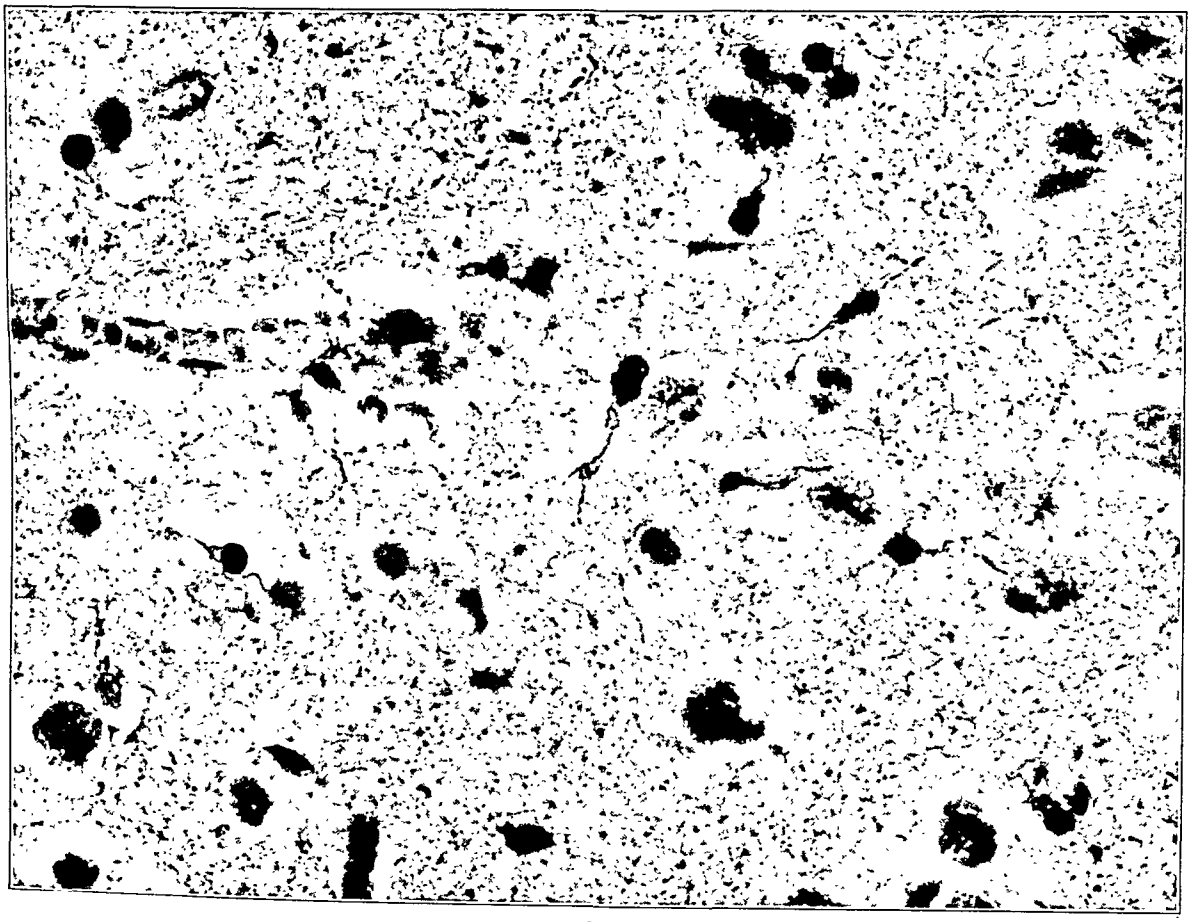
Plate 49

Fig. 5. Fibrillary astrocytes impregnated with pure silver diammino hydroxid and developed with buffered formalin; note the greater precision, the more delicate and truthful rendition of the fibrillae and the general uniformity of the background. Case 3.

Fig. 6. Oligodendroglia in the cortex of Case 1. These are the small, dark cells at the center of the field, with peculiarly shaped dendrites that tend to fan out at their extremities. One with four processes is seen at the right and somewhat below center. Impregnated with pure silver diammino carbonate (K. and D. method) and developed with buffered formalin.



5



6

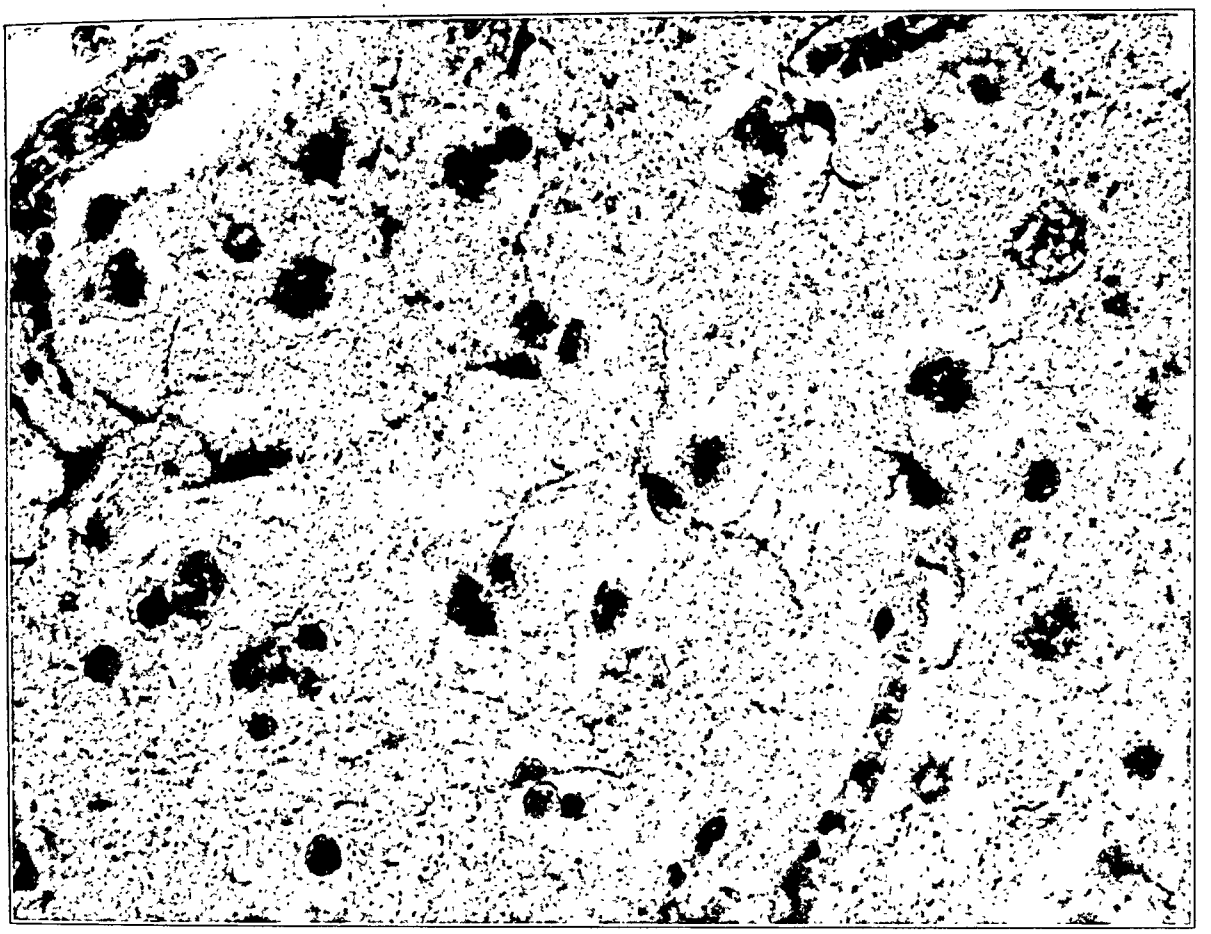
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Impregnation of Neuroglia

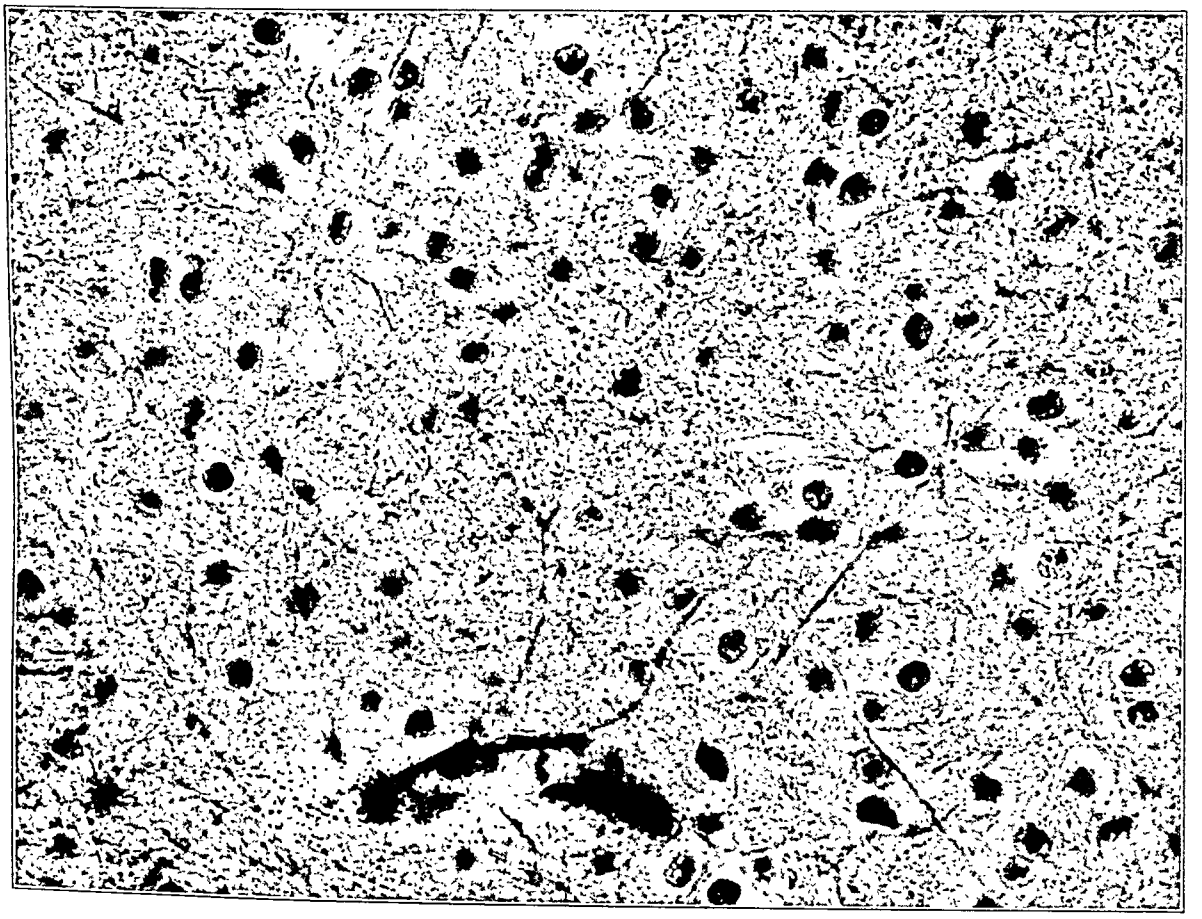
PLATE 50

FIG. 7. Microglia in the same case, impregnated in the same manner. Two, with their complex dendrites, are well shown at the center, two others attached to small vessels, one at the left, the other below near the middle. Others are less distinctly seen. In the upper right corner is an oligodendroglia satellite cell associated with a nerve cell; another, showing "acute swelling," is seen below the vessel at the left and resembles a rosette about a central plaque (the nucleus).

FIG. 8. The effect of strong buffer, 0.002 per cent sodium carbonate, in contrast to 0.0002 per cent in all the preceding pictures. Note the reappearance of the neurofibrillae, which were suppressed in the other cases. Indications of astrocytes are to be seen here and there, but there is great confusion of neuroglia and neurofibrillae. This is one of the sections impregnated by the Kubie and Davidson silver diammino carbonate solution in which the effect of an excess of sodium carbonate was tested out. Case 3.



7



8

Foot

Impregnation of Neuroglia

SILVER STAINING OF THE SKIN AND OF ITS TUMORS*

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Long known as a capricious staining substance, silver does not always deserve this reputation. Provided that certain conditions are fulfilled, silver staining becomes as simple and as certain as the staining of nuclei with hematoxylin; at least, this is true of the peripheral nervous system, of the skin, and of the argyrophil † reticula generally.

The conditions are: (1) suitable fixation; (2) suitable mordanting; (3) the use of silver solutions ten to twenty times stronger than those in vogue.

The fixative determines the result of the stain. When the silver worker has grasped this principle, half of his difficulties disappear. Differential silver staining might well be called differential fixation, for a given silver stain will produce quite different effects according to the fixative employed. This paper will deal with the application of these principles to the skin and to tumors of the skin, the fixatives selected being Bouin's fluid and neutral formol. The resulting preparations will be described separately, for these fixatives are not interchangeable.

BOUIN FIXATION

Figure 1 illustrates a paraffin section of normal skin that has been (1) fixed in Bouin's fluid; (2) mordanted with the Mallory bleach; (3) stained with del Río-Hortega's lithium silver made ten times stronger than the original formula.

The epithelial cells hold the stain, not only the surface epithelia but also those of the hair follicles, the sebaceous glands and the sweat glands. The staining of the epithelia is peculiar in that only the cytoplasm is colored; the nuclei are colorless. This is a constant feature of Bouin-fixed tissue, both normal and pathological.

There is a curious exception to the positive staining of the epithelia. The basal layer does not stain; it looks as if the epithelium had

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† Schiefferdecker's popular word *argentophil* is a hybrid. Professor Schaffer points out that the correct form is *argyrophil*, which he attributes to Merk, 1899.

separated from the corium, but counterstains show that the basal cells are in place. Unna noted the same thing in his permanganate stain for reduction points.

On examination of the corium beneath the epithelia, in the actual section the most striking feature is the reddish purple collagen with its fine graining, indicating the ultimate fibrillae. Here and there, traced in black, are the delicate reticula of the arrector pili muscles, of the walls of the vessels, of the sheaths of the sweat glands and of the nerve fibers. Only the high magnification of an immersion lens brings out the full beauty of these silver collagen stains, presenting the fine lines and sharp contrasts of an engraving.

Our first conclusion is that we have here a good collagen-reticulum stain. In truth, it is one of the shortest, simplest and most constant; but it is more than this. In contrast to the prominent epithelia, note that every cell native to the corium has vanished; fibroblasts, mast cells, endothelia of the vessels, smooth muscle cells of the vessels and of the arrector pili muscles, all are gone. When we recall that the epithelia are ectodermic and that the vanished cells of the corium are of mesodermic origin, we begin to perceive the selective possibilities of this technique.

Tumors

It is interesting to observe this differential staining of ectodermic and mesodermic cells continued in their progeny as they form tumors in the skin. The daughter cells are true to type. In tumors of mesodermic origin, fibromata, angiomata, lipomata and myomata, all cells disappear, leaving the reticula clearly outlined in black. In ectodermic tumors, adenoma, papilloma and epithelioma, the epithelial cytoplasm holds the stain, while the mesodermic cells of the interstitial tissue disappear; that is, the descendants of the fibroblasts disappear, lymphocytes remain, as will be related under sarcoma.

The sarcomas present a more complex problem, which will be discussed in a future paper. Still, workers wishing to experiment with this technique may be interested in our tentative conclusions in this none too well illuminated subject. Sarcoma, of course, is a mesodermic tumor, and one would say that if this technique is truly selective the sarcoma cells should be silver-negative. In the embryo, however, the mesodermic cells differentiate in two directions. One

line forms the framework of the body; these cells descend as fibroblasts, osteoblasts, vessel endothelia and, eventually, fibroblastic sarcoma and osteogenic sarcoma. With Bouin fixation, this lineage, from the simple fibroblast to the fibroblastic and osteogenic sarcoma, is silver-negative. In the other line, the mesodermic cells differentiate into blood- and lymph-forming tissues, red cells, bone marrow, spleen, lymphatic tissues, lymphocytes and leucocytes, continuing under favoring conditions as lymphosarcoma and sarcoma of bone marrow. In Bouin-fixed tissue, this lineage, from beginning to end, is silver-positive.

Holding rigorously to the principle that function determines structure, Menetrier long ago divided the sarcomas into these two groups. It is curious to see how this selective silver technique duplicates his functional grouping in every detail.

Of the Rous chicken sarcomas thus far examined, the cells are silver-positive, agreeing with Carrel's observation that they spring from macrophages and not from fibroblasts.

In sarcomas of every kind, giant cells are positive, as they are in tuberculosis, in nevi and wherever found.

Nerves and Nerve Tumors

The nerves of the skin present a special problem. With this technique, the axis cylinders, the neurokeratin of the myelin sheath, and the Schwann cells are constantly negative. This picture does not harmonize with Harrison's well known experiment which seems to prove that the Schwann cells are derived from the ectodermic neural crest. We may recall, on the one hand, that the basal cells of the epidermis are silver-negative and, on the other hand, that more than one keen observer has asserted the mesodermic origin of the Schwann cells (quoted by Cajal, page 203). The question cannot yet be regarded as settled beyond dispute.

On the peripheral nerves there occurs a puzzling tumor, known in this country as perineurial fibroblastoma, sometimes as neurosarcoma, and held to be derived from the fibroblasts of the nerve sheaths. Most European pathologists call it Schwannoma or peripheral glioma and derive it from the Schwann cells, the "peripheral neuroglia." The cells of this tumor are silver-negative, not throwing much light on their disputed origin; for both the Schwann cells and the fibroblasts are silver-negative also.

Formol Fixation

Figure 2 shows a paraffin section of the same skin fixed in 10 per cent neutral formol. Bearing in mind our maxim that the fixative determines the result of the stain, we should expect this fixative to produce a different result from Bouin; and so it does. As with Bouin fixation, the epithelia take the stain; but, in formol fixation, it is the nuclei that are black, while the cytoplasm is colorless. This epithelial nuclear selection is constant in formol-fixed tissue, both normal and pathological, from all parts of the body.

On examination of the corium, we seem at first sight to have the same picture as with Bouin, the mesodermic cells having vanished. On closer examination, we see that formol fixation makes a further differentiation in the corium. The endothelia of many of the vessels, the smooth muscle cells of the vessels and of the arrector pili muscles hold the stain, or, at least, their nuclei do, following the rule of formol fixation. This curious differentiation in the corium is frequent but not constant.

Tumors fixed in formol follow the general lines of Bouin fixation in differentiating between epithelia and mesoblastic cells and between structural and blood-forming mesoderm; but the results are not so constant as with Bouin fixation. The question merits further study.

Discussion

Of all the sharp eyes that have studied silver preparations, those of the Spanish master, del Río-Hortega, seem to have been the only ones to detect the differential staining of the epithelia and the connective tissue cells. In his paper on various methods for staining the connective tissue reticulum, using formol fixation, occurs this phrase:

"Los protoplasmas de las células epiteliales (especialmente epidérmicas), del hígado, suprarrenal, riñon, células cianófilas, etc., muéstranse difusamente teñidos, contrastando su tinción con la incolorabilidad de los elementos conjuntivos normales." The illustrious scientist of Madrid does not seem to have pursued the matter further.

The silver negativity of the basal epithelia and of the Schwann cells and the division of the mesenchyme into silver-positive and silver-negative lines show that the principle of embryonic origins

cannot be applied too strictly. Function rather than embryological origin may determine the reaction; in fact, if we exclude collagen, a feeble reducer, the reaction of the cells to silver parallels the reduction staining so thoroughly studied by Unna. Whatever the explanation, it seems that when, in the course of its development, a cell has acquired a positive or negative silver relation, its retention of that quality through many pathological vicissitudes enables us to recognize that cell when found far from its natural habitat and in activities that seem foreign to its nature.

Technique

1. Fix in Bouin for 3 days or in 10 per cent neutral formol for 3 days. Old formol material may be improved by immersion in fresh neutral formol for 3 days. Formol-fixed tissue immersed in Bouin for 3 days will give nearly perfect but not quite perfect Bouin pictures; a positive endothelial or smooth muscle nucleus here and there betrays the original formol fixation.

2. Embed in paraffin or celloidin; or make frozen sections.

3. Stick paraffin sections on the slide with Masson's gelatin glue and harden in hot formol fumes; sections so treated seldom float off.

4. After removal of the paraffin, wash Bouin sections in running water for 20 minutes to remove the picric acid; wash formol sections for 5 minutes.

5. Mordant with the Mallory bleach. Tissue recently fixed in formol (2 to 10 days) often gives the reaction without mordanting, but not constantly.

(*a*) 1 per cent tincture of iodin, 3 minutes; rinse in tap water.

(*b*) 5 per cent hypo (sodium thiosulphate), 3 minutes; rinse in tap water.

(*c*) ¼ per cent potassium permanganate, 3 minutes; rinse in tap water.

(*d*) 5 per cent oxalic acid, 3 minutes; wash well in running water for 10 minutes.

6. Distilled water; change 3 times within 5 or 10 minutes to ensure clean slides entering the silver solution.

7. Río-Hortega's lithium silver augmented to 10 per cent. Heat the stock solution in the oven to 50° C and stain in the oven

for 5 minutes. We filter the used solution into a glass-stoppered bottle and use it a dozen times or more.

8. Rinse the slides by pouring distilled water over both sides.

9. Formol, 1 per cent in tap water; flood the sections frequently for 3 minutes.

10. Rinse both sides of the slide with distilled water.

11. Yellow gold chlorid, 1 to 500 of distilled water in a Coplin jar; immerse the slides at room temperature for 10 minutes. The gold solution may be used many times.

12. Rinse both sides of the slide with distilled water.

13. Oxalic acid 5 per cent; pour on slide and leave for 10 minutes.

14. Rinse with distilled water.

15. Hypo, 5 per cent; pour on slide. Change as often as it becomes turbid for 10 minutes.

16. Wash well in running water. Counterstain if desired and mount in balsam.

The times advised are those that have given us the clearest pictures in the shortest time, but this is not a finical technique; the time of the different steps may vary widely without affecting the result. The Mallory bleach solutions and the formol may be left on the slides for an hour or more without harm. Even the silver solution may vary in strength from 2 per cent to 15 per cent, the time in silver from 2 to 10 minutes and the temperature of the silver solution from room temperature (20 minutes) to 60° C; though the longer times and the higher temperatures are likely to give precipitates and dirty slides. After the formol, the slides may be left overnight in gold or in oxalic acid; and at any point in the technique the slides may be left overnight or over Sunday in distilled water or in tap water. In fact, some of us believe that counterstaining is better if the slides are left in tap water overnight between the hypo and the counterstain.

Historical Note

The use of potassium permanganate followed by a reducer entered histological practice in 1885, when Lustgarten borrowed it from the dyeing industry. In the next year Pal adopted it in his modification of Weigert's myelin stain, changing the reducer from sulphurous acid to a mixture of oxalic acid and potassium sulphite.

In both instances the reaction was used after staining to differentiate overstained tissue.

The use of permanganate and oxalic acid as a mordant before staining begins with Weigert's neuroglia and fibrin techniques in 1895. Against much opposition, Weigert remained convinced of the necessity of "oxidation and reduction" as a preparation for staining neuroglia and all other tissue difficult to stain. In 1898, Rosenthal applied the new Weigert neuroglia technique to a Zenker-fixed tumor and necessarily preceded the bleach with iodin-alcohol extraction of the mercury. In 1900, Mallory included the permanganate-oxalic acid mordant in his phosphotungstic acid neuroglia stain. In 1908, he changed the fixative to Zenker and established the bleach as it has been used ever since in American laboratories to prepare Zenker sections for staining.

All silver stains for reticulum go back to del Río-Hortega, Maresch and Bielschowsky; but mordanting with permanganate and oxalic acid was not part of the original technique. It begins with Perdreau, in 1921, who mordanted formol sections with permanganate and Pal's decolorizer. In 1924, Foot advised the full Mallory bleach for paraffin sections of Zenker material. In 1925, del Río-Hortega published his Variant C for reticulum, mordanting frozen sections of formol material with permanganate and oxalic acid; he mentions Biondi as using it in his adaptation of Cajal's gold technique to connective tissue.

The writer's contributions are the use of the Mallory bleach as a mordant for Bouin-fixed tissue, the increase of lithium silver to 10 per cent (indispensable for the reticulum on nerve fibers), and the intercalation of oxalic acid between the gold and the hypo.

Formulas

Bouin's Fluid (Masson's Formula): To 300 cc. tap water add 100 cc. commercial formol and 20 cc. glacial acetic acid. Add an excess of picric acid. Shake frequently and keep an excess of picric acid in the fluid. Ready for use in 3 days and keeps indefinitely.

Masson's Gelatin Glue: Dissolve 0.05 gm. gelatin (in practice, a bit of ordinary sheet gelatin 5 millimeters square) in 20 cc. distilled water, warming it over the flame. Place a row of slides on the warm plate. Filter a large drop of gelatin solution on each slide and float

the paraffin section on it. As soon as the section spreads, stand the slide upright to drain, holding the section for a moment in the desired place with a brush or needle. At this stage do not permit the section to dry; when the excess gelatin has drained off, blot the section and place immediately in the oven at 45 to 50° C in formol vapor, secured by leaving in the oven an open dish of formol. For staining with hematoxylin and anilin dyes, 20 minutes in the hot formol vapor is sufficient; for silver staining, leave the slides for several hours or, better, overnight.

Ten Per Cent Lithium Silver (Modified del Rio-Hortega): To make 120 cc. In a 250 cc. glass-stoppered graduate, dissolve 12 gm. silver nitrate C. P. in 20 cc. distilled water.

Add 230 cc. saturated solution lithium carbonate C. P. in distilled water; shake well; let settle to about 70 cc. of precipitate; wash well with distilled water 3 or 4 times.

After settling to about 70 cc. of precipitate, decant the wash-water; add aqua ammonia fortior, shaking constantly, until the fluid is almost clear.

Add distilled water to a total of 120 cc.; shake and filter into stock bottle. The solution keeps for many months. It is so strong that a slight precipitate is negligible.

Ordinary filter paper is apt to turn brown and discolor the solution while filtering. Use Whatman filter paper No. 42 or No. 44 or Schleicher and Schüll No. 589.

It is a pleasure to thank the many friends who have given the material for these observations, among them Professor Clarke, Director of the Laboratory, Professor Stout and Dr. Virginia Frantz; Professor Jobling and Dr. Kesten, of the Department of Pathology; Professor Hopkins, Dr. Machecek, of the Department of Dermatology; Drs. Carrel and Ebeling, of the Rockefeller Institute; and Dr. Stewart, of the Memorial Hospital. The writer is especially indebted to Professor Ewing, who has given generously of his specimens, of his time, and of his rich experience.

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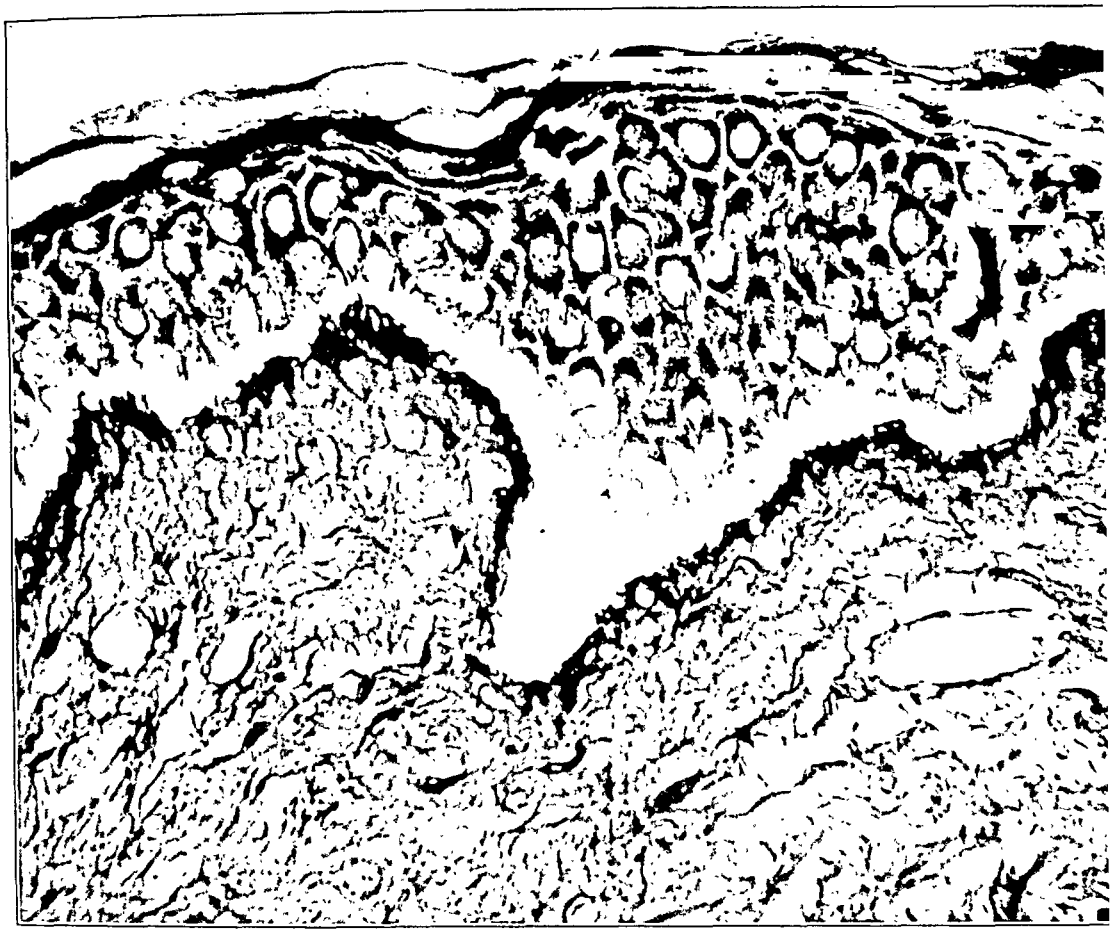
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DESCRIPTION OF PLATE

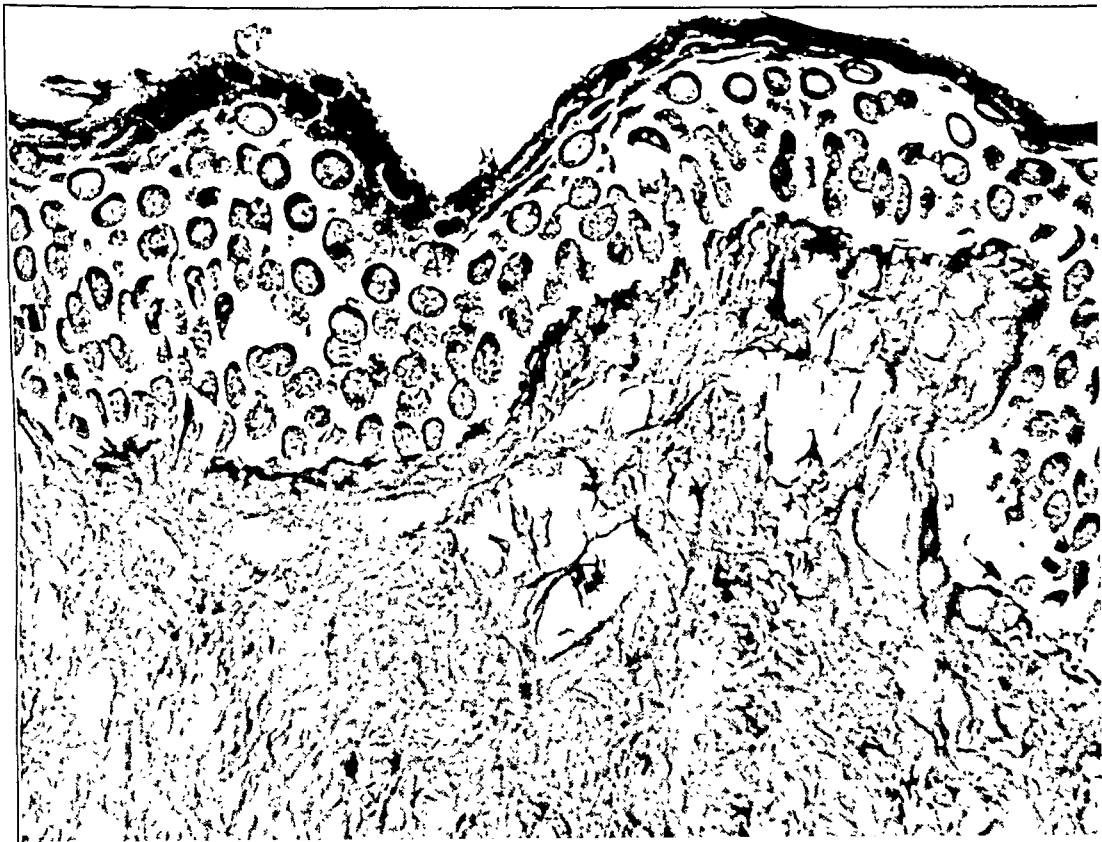
PLATE 51

FIG. 1. Normal skin fixed in Bouin. Epithelial nuclei and basal cells colorless; mesodermic cells of the corium colorless. × 500.

FIG. 2. Normal skin fixed in neutral formol. Epithelial nuclei positive, cytoplasm colorless; mesodermic cells of corium colorless. × 500.



I



MECONIUM ILEUS WITH CONGENITAL STENOSIS OF THE MAIN PANCREATIC DUCT*

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This report concerns a case of intestinal obstruction in a newborn, a double perforation of the ascending colon with a superimposed peritonitis and the formation of a false diverticulum in the ileum, as a result of accumulated thickened meconium. Very few isolated cases have been reported in the literature (Landsteiner,[1] Fischer,[2] Fanconi[3]).

REPORT OF CASE

Clinical History: R. C., a female infant, was born at full term in a primipara after a normal gestation and normal delivery. The birth weight was 7 pounds, 3 ounces. The child was breast fed six times a day at three hour intervals and was given water between feedings. For two days the child progressed well with the exception that no meconium passed. On the third day a moderate amount of distention was noted and the child vomited 20 minutes after feeding. During the third and fourth days, the distention became progressively more marked and the vomiting continued. Milk appeared in the mother's breast on the fourth day. The infant was admitted to the service of Dr. Charles Elsberg on the fourth day because of progressive distention of the abdomen, continued vomiting for two days and failure to pass meconium since birth.

Examination: This showed an acutely ill, drowsy, female infant with a markedly tympanitic, distended abdomen, over which the skin was tense and streaked by a number of prominent veins. The umbilical stump was clean and protruded as a black cord. The rectal examination was negative. There were no external congenital abnormalities. Temperature 98°, pulse 170.

Course: Roentgenographic examination of the abdomen revealed the presence of free air in the peritoneal cavity with marked distention of the small intestine (Fig. 1). A diagnosis of intestinal perforation due to intestinal obstruction was made and the abdominal cavity was explored by Dr. P. Klingenstein. Upon incising the peritoneum there was a release of gas. The loops of the small intestine were markedly distended and covered with a pasty brownish material. There were a number of distention ulcers and a Meckel's diverticulum which showed no inflammation. The exploration was discontinued because of the child's poor condition and drainage was instituted to the region of the cecum, where the greatest amount of reaction appeared.

The postoperative course was extremely poor. The child repeatedly vomited small amounts of fecal-appearing material. A Murphy infusion was expelled. The child's condition became progressively worse and death ensued seventeen hours postoperatively, three days after the onset of symptoms.

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Pathological Report

A necropsy was performed by Dr. Klemperer and Dr. Kornblith seventeen hours after death. An abstract of the protocol reads as follows:

The body is that of a well developed, white, 5 day-old infant in incomplete rigor mortis. The umbilical cord appears as a 4 cm. black protrusion on the abdomen. There is no reaction about the base of the umbilical stump. The abdomen is tremendously distended. There is a 5 cm. lower right rectus incision. At the lower angle of the wound, there is an ill-defined tract extending down to the region of the cecum. On opening the peritoneal cavity many loops of intestine are found loosely adherent to the anterior parietal peritoneum. Agglutinations are present about all the loops of intestine. There are no abnormal bands; no volvulus or kinking of the gut. In the peritoneal cavity about 100 cc. of brownish green, fecal-appearing material is found either loosely or in pockets closed off by intestinal loops. The same material is present in the right lumbar gutter. There are fibrinous adhesions between the liver and the diaphragm, as well as between the spleen and the parietal peritoneum.

Gastro-Intestinal Tract: The oesophageal mucosa is reddened and has a brownish, sticky, mucoid material on its surface. The stomach is moderately dilated and contains about 15 cc. of a loose, mucoid, brown, non-fecal-smelling material. The mucosa is edematous. The duodenum and jejunum are extremely distended to about six times the diameter of the normal lumen. Throughout its length, the intestinal lumen is filled with meconium which is like putty in consistency. It is very tenacious and adheres very firmly to the mucosa, from which it can be separated only with difficulty, leaving a smooth surface. This same condition obtains in the proximal ileum, which in addition shows five ulcers on the antimesenteric border measuring 2.5 to 5 mm. in diameter. The ulcers are flat and extend through the mucosa and muscularis. Two of the ulcers actually perforate the serosa. At 27 cm. from the ileocecal junction, there is present a true Meckel's diverticulum, which is conical in shape, about 1 cm. high and 1 cm. across the base. It possesses all the coats of the intestine and shows no inflammatory changes about it. Ten cm. distal to the diverticulum, *i.e.*, 17 cm. from the ileocecal junction,

there is a defect in the serosa and muscularis which appears as a wedge-shaped, clear-cut area, through which there protrudes the mucosa of the ileum as a false, conical-shaped diverticulum about 1 cm. high and 1 cm. across the base. It is tissue paper-thin and breaks on manipulation. There are fibrinous adhesions about it. The ileum, from the Meckel's diverticulum to the cecum, feels thickened. On the mesial aspect of the ascending colon, about 1.5 cm. from the ileocecal junction, there is a collection of brownish, granular material, partly walled off by adhesions. The wall of the ascending colon at this point shows a perforation 1.5 cm. in diameter. The edges of this perforation are everted. The mucosa protrudes through the opening in the serosa and muscularis. One cm. distal to this area on the ascending colon there is a similar perforation. The proximal half of the colon is considerably distended. Just beyond the hepatic flexure it becomes collapsed. The area of transition is not abrupt. It extends over a distance of 2 cm. without any zone of demarcation. Here the tenacious brown intestinal contents are looser in consistency. Distally the colon is completely collapsed. It contains a fairly thick mucoid colorless material which bears no resemblance to meconium. The mucosa is untinged. The wall of the intestine is edematous throughout. In the region of the rectosigmoid, there is a diffuse, submucosal hemorrhage for a distance of 2 cm. Nowhere is there any anatomical obstruction to the lumen of the gut (Fig. 2).

Other Tissues: The lungs are markedly congested and atelectatic. The heart shows a patent foramen ovale. The liver shows no abnormality. The gall-bladder contains a small amount of bile. The common duct can be probed without difficulty. The spleen is negative. Both kidneys show uric acid infarcts. The adrenals are both large. On cut section, the zona reticularis is practically intact, except at the lower pole, where it shows beginning hemorrhage.

Pathological Diagnoses: Meconium ileum; generalized meconium peritonitis; perforations of the ascending colon; perforation of a false diverticulum of the ileum; Meckel's diverticulum; atelectasis of the lungs; uric acid infarcts of the kidneys; status after exploratory laparotomy for intestinal obstruction.

Histological Examination

Sections taken from all parts of the intestinal tract for histological study reveal the following.

Mucosa: The mucosa of the entire small intestine shows no abnormality except in the region of the terminal ileum. Here it is very much flattened. At the sites of the tension ulcers, the mucosa is absent. At the false diverticulum, the outpouting mucosa is necrotic and contains a few lymphocytes and a number of bacteria. The mucosa of the entire colon is also markedly flattened out, especially in the section of the collapsed descending colon.

Submucosa: The submucosa throughout shows marked congestion with small hemorrhagic areas at irregular intervals and marked edema which extends through the major portion of the stroma.

Circular Muscle: The circular muscle is widened throughout the course of the small intestine except at the tension ulcers, where the muscle fibers are narrowed, hyalinized and necrotic. In the ascending colon the muscle fibers are compressed to about one-half of the diameter of the circular muscle in the descending colon, which appears normal. Throughout the intestine there are wide gaps in the muscularis through which the nutrient vessels penetrate to the submucosa. These gaps occasionally measure as much as the entire width of the intestinal muscle itself.

Outer Longitudinal Muscular Layer and Serosa: These show marked edema. On the surface of the serosa there is a thin coat of a homogenously pink-staining amorphous substance and in places a thready granular material containing some fat and bile pigment—apparently adherent meconium.

Leucocyte Reaction: There is no polymorphonuclear leucocytic reaction in any part of the intestine, although there is an increase of mononuclear cells within the intestinal wall at the site of the tension ulcers. However, both gram positive and gram negative bacilli and cocci of various types are present upon the serosa.

A section through the tension ulcer shows an abrupt interruption of the mucosa. The submucosa is hemorrhagic and its structure is unrecognizable. The muscularis contains a few bacilli and a number of mononuclear infiltrations.

Nerves: The sympathetic ganglia around the celiac axis stand out clearly and show no pathological changes. The plexuses of Auerbach

and Meissner appear normal in all sections. In the mesentery of the descending colon nerve bundles stand out prominently, again appearing normal.

Pancreas: The pancreas shows a marked increase in the stroma. The acinar tissue is in general atrophic and in many areas is completely replaced by connective tissue. The ducts are dilated and show extensive proliferation. These changes are especially evident in the head of the pancreas. The islands of Langerhans, however, are apparently unaffected (Figs. 3 and 4). The main pancreatic duct is extremely widened and contains a granular pink-staining substance in globules or concretions which fill the lumen (Fig. 5). The dilatation of the ducts extends even into the terminal portions involving the acini with the result that the acinar cells are flattened, occasionally resembling the small ducts (Fig. 5). There is no evidence of any reaction about the duct except in the body of the pancreas, where a small amount of lymphocytic infiltration is present.

Meconium: On microscopic examination the meconium shows many round, hazy, fat droplets which stain with Sudan III, desquamated epithelial cells, and a few cholesterol crystals. There are very few gram positive, large diplobacilli which are probably contaminants.

Umbilical Stalk: This shows no changes.

Discussion

In reviewing the findings, it must be noted that this is a case of ileus, with intestinal perforations, a consequent peritonitis and fibrotic changes in the pancreas, in an otherwise relatively normal 5 day-old infant.

Did this perforation occur before birth, during birth or after birth? In four reported cases of intestinal perforation where the perforations were traced back to the time of delivery, the symptoms of distention, of vomiting, or both were present immediately after birth or when feeding was attempted for the first time. Death invariably supervened within fifteen hours (Zillner [4]). From the gross and microscopic studies it is seen that the peritoneal exudate is in the nature of a recent reaction. There is no organization. There are agglutinations but no adhesions. The meconium in the peritoneal cavity appears fresh, not calcified, and there are no foreign body giant cells.

The infant was apparently well for two days before the onset of clinical symptoms and died as late as the fifth day. It is, therefore, most likely that the perforation occurred during the third day at the time of onset of the vomiting and distention.

The nature of the perforation is described almost identically by all authors. It is a perforation about 0.7 to 1.5 cm. in diameter, whose edges roll outward, showing a pouting edge of necrotic mucosa and submucosa (Zillner). It is agreed that such a picture is consistent with a "blow-out" from within the lumen by increased pressure at a point of weakness in the muscularis and serosa (Genersich [5]).

The position of the two perforations in the colon is in accord with the site of predilection for saccule or false diverticulum formation as conceived by Drummond,[6] and others. Both occur on the anteromesial side of the ascending colon in the region of the haustra between the tenia mesenterica and tenia libera at a point of entrance of the blood supply to the intestine, a point of anatomic weakness. Progressive distention of the ascending colon present in this case would produce the necessary and sufficient conditions for the formation of a diverticulum. The diverticulum thus formed is more prone to necrosis and perforation, since by passing through the region of its blood supply its own nutrition is compromised. Rupture may then be expected to occur. By their gross appearance and microscopic study the perforations may be considered as ruptured false diverticula.

The diverticulum in the ileum does not conform to the pathological picture in any stage of a tension ulcer, since in the latter the changes take place first in the mucosa and submucosa. Since the gross appearance shows a clear-cut edge like that of an incision of the serosa and no changes in the intestinal wall, this diverticulum on the antimesenteric border conforms more to an injury to the serosa and muscularis, produced from the outside during manipulation of a distended loop of gut at the exploratory laparotomy. The distention continued and at this weak point the mucosa protruded. The outpouting mucosa in the perforations of the ascending colon, however, must be considered as pulsion diverticula going through an anatomically ill-defended space like a hernia through the internal inguinal ring (Drummond). A similar condition is found in Fischer's case.

This case cannot be classified among those of so-called spontane-

ous perforation due to injury as assumed by Zillner. There is no history of trauma to the mother or to the child at birth. Also, such spontaneous ruptures reported are all single, occurring in the sigmoid. And finally, the hypertrophy at the ileocecal region as well as the tension ulcers in the ileum indicate a condition of some duration and not an acute spontaneous occurrence. A primary inflammatory lesion of the intestinal tract is not to be considered. It is most likely that organisms played no causative rôle in this condition. There is no evidence of an inflammatory reaction due to organisms anywhere, even in the region of perforation. The peritoneal exudate too was not foul-smelling, and the meconium was singularly free from organisms. Kendall [7] reports the meconium sterile at birth and demonstrates organisms as invaders on the third day. The pathogenesis of this case agrees more with the conception of Paltauf,[8] who pictures the reaction as one of necrosis due to circulatory interference. The microscopic sections confirm this view.

Hypoplasia of the large intestine is out of the question (Fanconi). The gross appearance and microscopic sections show no such changes. In addition, the collapse would be expected to begin at the ileocecal junction rather than at the transverse colon and very little material to have advanced through the ascending colon. The condition here is obviously different.

It is highly improbable that there was any neurogenic disturbance here causing intestinal stasis. Keith [9] believes that irritation of the neuromuscular element, or Auerbach's plexus produces spasticity of the intestine and that its destruction causes loss of tonus and stasis. He was able to demonstrate the presence of definite fibrotic and degenerative changes in this nodal tissue in segments of large intestine extirpated for the relief of intestinal stasis. Examination of the sympathetic ganglia and myenteric plexuses reveals no changes in this case. They stand out clearly as normal structures in both the dilated ascending colon and collapsed descending portion. There is, in addition, evidence that there had been intense peristalsis in the large intestine, in the marked dilatation, diverticulum formation and progression of meconium to the hepatic flexure.

The history of swelling of the abdomen, vomiting and retention of meconium gives the cardinal symptoms of obstruction. The common causes for obstruction, such as stenosis, fetal bands, atresia, kinks, intussusception, volvulus, are absent here. The postmortem

findings indicate no anatomic block to the intestinal lumen, and yet there is a collapse of the large gut at the hepatic flexure and the remainder of the mucosa of the colon is untinged by meconium. This speaks for an obstruction from within, especially since at birth meconium should have been down near the anus. In the normal condition meconium reaches the ileocecal junction during the fourth month and the rectum by the fifth month of pregnancy. It passes quickly along the jejunum, and at birth the entire large intestine and the ileum are distended with it (Torkel [10]). In this case the meconium was found putty-like in consistency, fatty and extremely tenacious, and had progressed no farther than the hepatic flexure. It was thickest at the region of the ileocecal junction and cecum, and became progressively less thick oralward. The obstruction is, therefore, attributed to the thickened meconium.

It is clear that there was obstruction and that the obstruction was in the lumen. Again this obstruction must have occurred at two points: the first at the ileocecal junction and the second, a later one, at the hepatic flexure. It is important to note that the type of intestinal content and its disposition determine the hypertrophy and dilatation of the intestine. Where the content is loose, more can fill in the lumen without impaction and efforts to expel it will cause dilatation as well as hypertrophy. This is illustrated in a case of jejunal atresia, in which the proximal jejunum contained loose granular material, was dilated to the size of an infant's arm, and showed hypertrophy of its wall after six days (Fanconi). Again, when the material is hard and the time of stasis is short, dilatation will take place first without hypertrophy. While there is hypertrophy and dilatation and tension ulcer formation in the distal ileum in the case reported, dilatation alone without hypertrophy is seen in the ascending colon. This points to the large intestine as the region of most recent stasis.

If the obstruction then was the result of inspissated meconium, it is now proper to investigate the cause of the meconium thickening. A case of Torkel's showed infiltration and hyperemia in the intestinal wall. He attributed the thickening of the meconium to the inflammation of the intestinal wall. Fanconi offers the suggestion that there may be an interference with the normal secretion of the intestinal glands. Farrer's case in Fanconi's series showed an obstructive jaundice, and he believed that the insufficient secretion of bile

resulted in a changed consistency of the meconium. Landsteiner's case demonstrated a fibrosis of the pancreas to which he attributed the thickening of meconium. The case here reported shows an identical change.

The cause of the pancreatic fibrosis was next investigated.

It is known that syphilis can produce similar changes of fibrosis in the pancreas, however, without such marked dilatation of the duct, which is an outstanding pathological change here. The organs showed no signs of syphilis, and a thorough search in Levaditi preparations revealed no spirochetes.

It may be proposed that the connective tissue skeletal structure and the parenchyma in their development, by proliferating at different rates, will result in fibrosis and atrophy of the secreting portion. This, however, again would not account for retained secretion and dilatation of the ducts.

Serial sections through the head of the pancreas, over 600 in all, were studied with special reference to the ducts. The lumen of the duct of Wirsung was enormously dilated throughout its course in the body and tail, but it was found to decrease in size when it reached the head of the pancreas 600 microns from the duodenal opening. Its wall was thrown into folds and around it were proliferated ducts, similar to those present in the periportal spaces of the liver in cases of congenital atresia of the common bile duct. At the region of narrowing, the epithelium was absent, and the duct was recognized with difficulty as a microscopic slit. There was, however, no atresia, and the lumen was observed to widen only slightly on its way from the zone of greatest narrowing to the duodenum. The duct of Santorini could be followed for only a short distance. It ended in the tissue of the head of the pancreas, showing no outlet into the duodenum (Figs. 6 and 7).

The changes in the pancreatic tissue were identical throughout the organ as described above, namely: marked fibrosis, distention of the ducts with retained secretion, distention of the terminal ducts and atrophy of the acini.

The incomplete course of the duct of Santorini is not extraordinary. Opie [11] found that the accessory duct though well developed, showed no opening into the duodenum in twenty-one of one hundred cases studied. However, there is not a single reference in the literature to the stenosis or atresia as an anomaly of the duct of Wirsung.

Yet, this is the condition here — a congenital stenosis of the main pancreatic duct.

The changes, present here correspond almost exactly to those brought about experimentally after ligation of the pancreatic duct. Ukai [12] has demonstrated conclusively that fibrosis of the pancreas and dilatation of the ducts may begin as early as four days after ligation of the ducts. It is suggested, therefore, that the stenosis of the duct of Wirsung in this case may have produced the changes described. For the cause of the stenosis, one may consider the theory advanced by Tandler [13] in explanation of duodenal atresia.

The major number of atresias of the intestine occur in the region of the duodenum. Tandler states that the duodenum is especially predisposed to atresias because of adhesion of its epithelium, *Epithelverklebung*, during the rotation of the gut in the embryo. It is normal for the epithelium to become agglutinated, and its failure to reopen causes an atresia.

There is no clue to the pathogenesis of the pancreatic duct stenosis, but it is possible to conceive it as a condition of *Epithelverklebung*, similar to that causing duodenal atresia as believed by Tandler.

The pathological picture of the pancreas is thus consistent with the blocking of the main duct with obstruction to the outflow of pancreatic secretion. Whether or not the absence of pancreatic secretion has any effect upon the consistency of the meconium must be considered from the following facts. Hess,[14] by means of the duodenal bucket method, showed that all of the three ferments, trypsin, steapsin and amylopsin, are present in infants before they are put to the breast. Meconium ordinarily contains mucus, bile, epithelial cells, fat globules, cholesterol and intestinal secretions. The water content of meconium is 72 per cent (Davy [15]). In contrast, a normal child's stool contains 84 per cent of water. The meconium is thus more concentrated in solids. The functions of the pancreatic secretion, as elaborated by Pflüger [16] and recently by Okada [17] and also Lombroso,[18] include digestion of fat and aiding in the absorption of the split products of proteins and fats from the intestine; and here may be added the dilution of the intestinal content. Cases with diminution of pancreatic function in Okada's series showed voluminous stools. Thaysen [19] reports Lombroso's experiments with ligated pancreatic ducts in dogs, which also showed the formation of fatty stools. In a newborn with multiple intestinal

atresias, the intestinal content was found crumbly and thick in a portion of intestine which had no pancreatic juice or bile accessible (Fanconi).

The absence of the pancreatic secretion normally found in meconium may be considered to have resulted in an alteration in its consistency, due to failure in dilution and failure in the digestion of fats and proteins present.

The submucosal hemorrhage in the sigmoid is an incidental finding and most likely due to trauma of rectal examination and insertion of a catheter. Landsteiner reports a similar finding in his case. In investigating injuries to the rectum and colon from below, Zillner attempted to introduce catheters in newborn infants and found it most difficult, so that at most a 5 mm. wide catheter went a distance of 6 cm. at most and caused injury in the rectum on its way.

The sequence of events then is considered to be as follows. Because of pancreatic insufficiency, a thickened meconium resulted in the infant's intestine. On its way through the intestinal tract it was obstructed at the ileocecal junction, a point of physiological narrowing. This caused intestinal peristalsis, hypertrophy of the ileocecal region and a back pressure with the production of tension ulcers in the distal ileum. Some of the thickened meconium was apparently driven through to the ascending colon after birth when feeding induced increased peristalsis. In the region of the hepatic flexure, another zone of physiological narrowing was encountered, resulting in complete obstruction. The cecum became distended, and two diverticula formed. Because of further peristalsis and increased pressure from the first two days feeding, the diverticula ruptured into the peritoneum on about the third day. Meconium peritonitis followed and caused the infant's death.

It is remarkable that only one other case is reported, that of Landsteiner, which is in every respect identical with this one, although the case of Fischer, reported recently, might very well be explained on a similar basis.

Summary

1. A case is presented with the rare anomaly of congenital stenosis of the duct of Wirsung.

2. The absence of pancreatic juice caused inspissation of meconium with subsequent intestinal obstruction, ileus and perforation.

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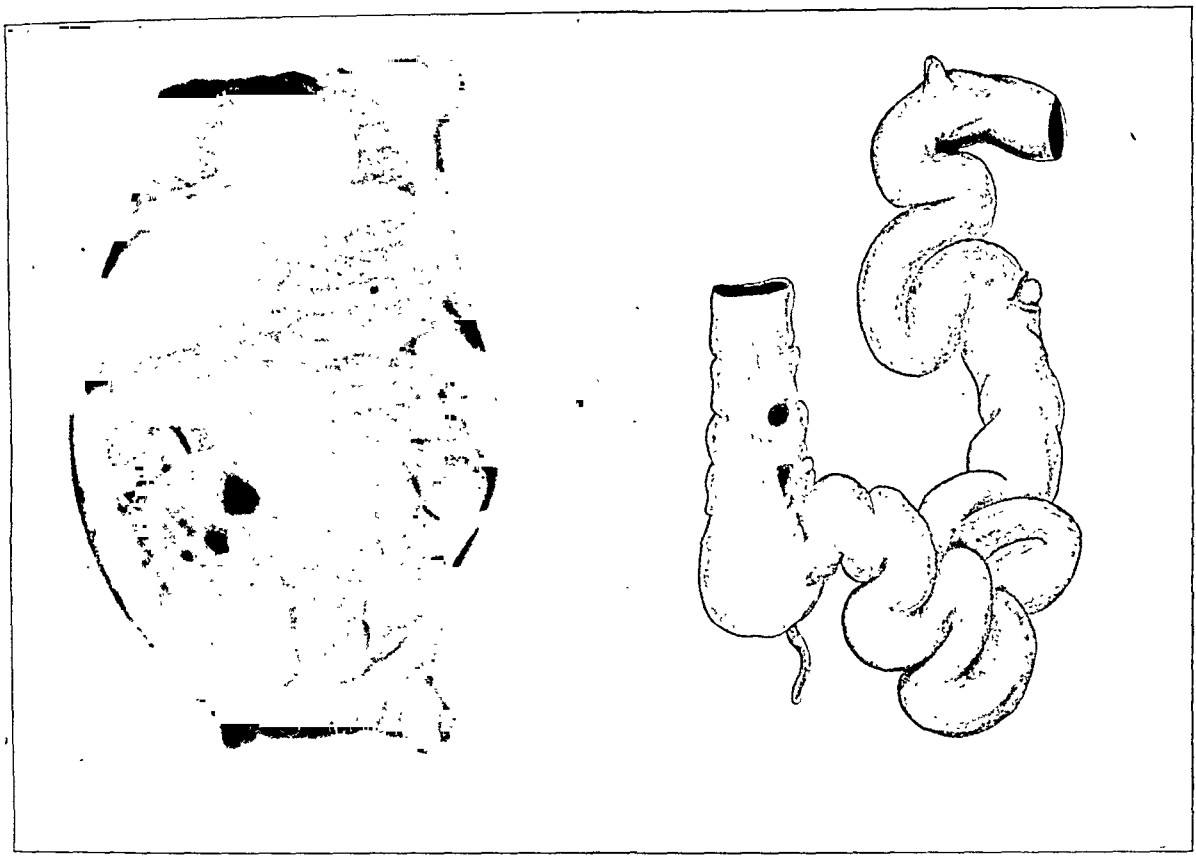
DESCRIPTION OF PLATES

PLATE 52

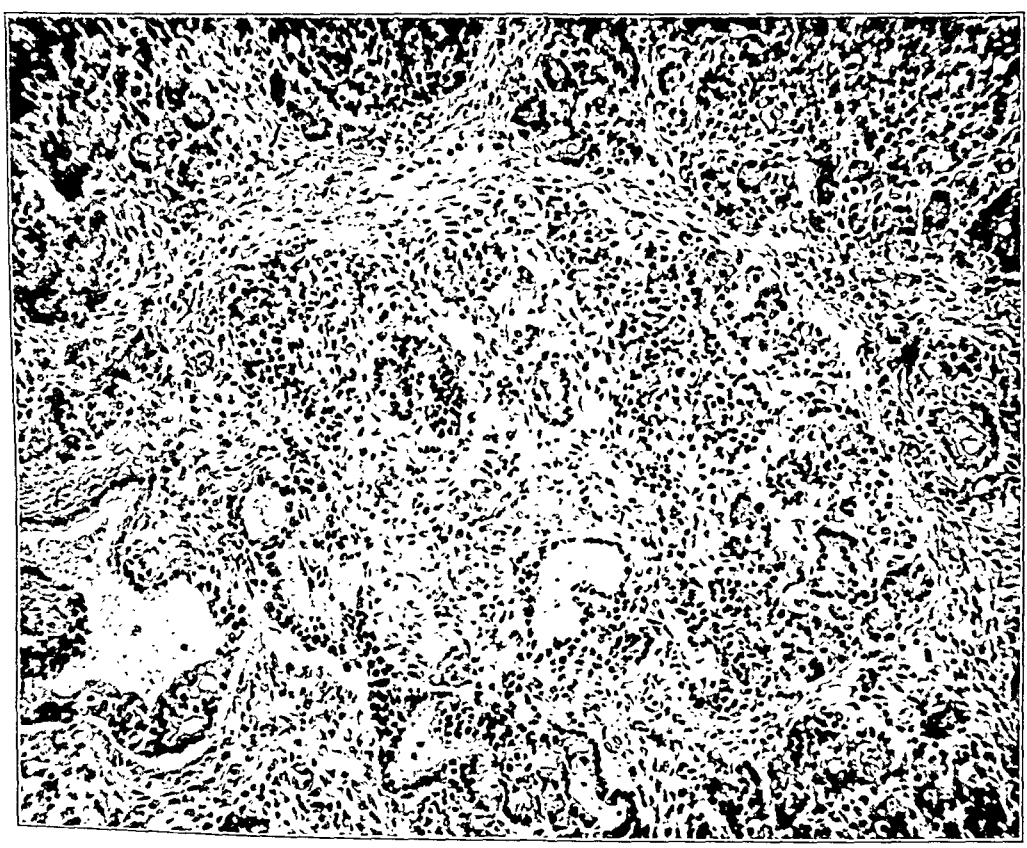
FIG. 1. X-ray of abdomen before operation, showing marked distention of the intestinal loops and free air beneath the diaphragm.

FIG. 2. Schematic drawing of terminal ileum and ascending colon, showing a Meckel's diverticulum, a false diverticulum of the ileum and two perforations of the ascending colon.

FIG. 3. Pancreas, showing increased connective tissue and atrophic acinar tissue with proliferation of the ducts. × 150.



1 2



3

Kornblith and Otani

Meconium Ileus

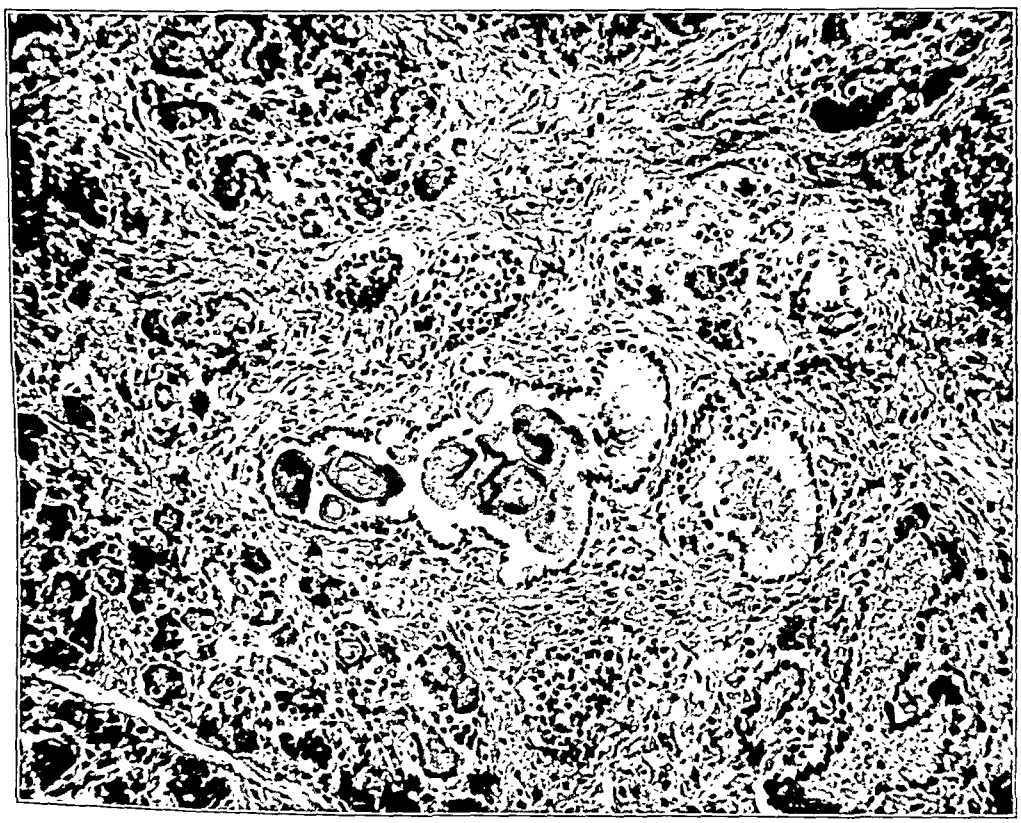
Plate 53

Fig. 4. Pancreas, showing marked proliferation of the smaller ducts and atrophic acini. × 150.

Fig. 5. Pancreas, showing dilated pancreatic ducts with concretions. Note the dilatation of the terminal ducts. × 150.



4

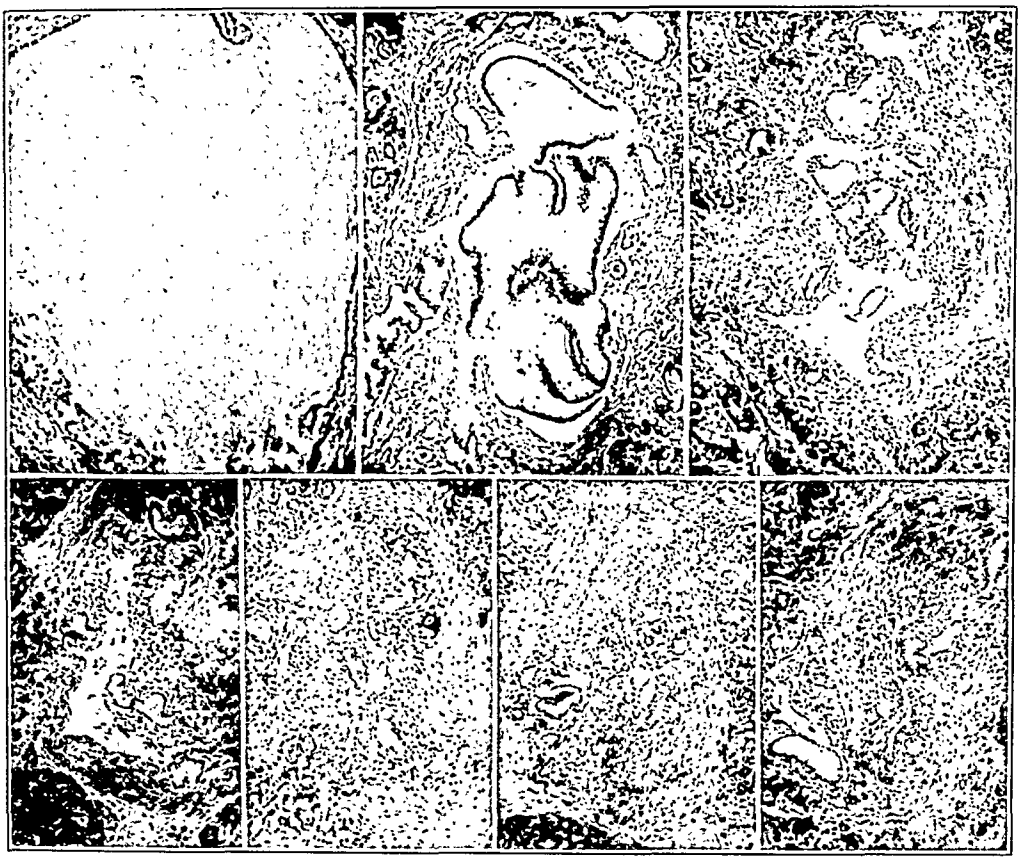


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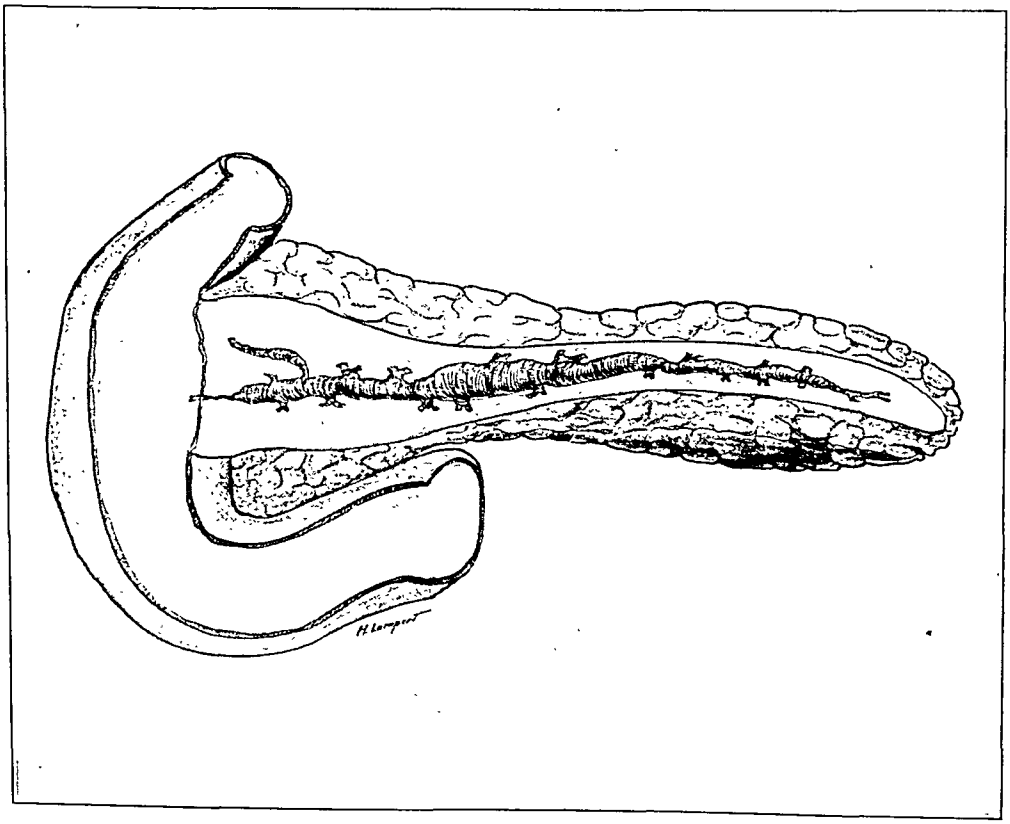
PLATE 54

FIG. 6. Pancreas, showing serial sections of the duct of Wirsung to illustrate progressive narrowing: (1) body; (2) body near head; (3, 4, 5) head; (6) narrowest portion; (7) terminal portion at duodenum. × 120; all of equal magnification.

FIG. 7. Schematic drawing of pancreatic ducts, showing stenosis of the duodenal portion of the duct of Wirsung with extreme distal dilatation.



6



7

Kornblith and Otani

Meconium Ileus

THE PERCENTAGE OF THE DIFFERENT TYPES OF CELLS IN THE MALE ADULT HUMAN HYPOPHYSIS *

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INTRODUCTION

The interest now taken in more precise investigation of the endocrine organs in abnormal conditions demands a corresponding increase in the knowledge of the normal variability of these structures. As rapidly as possible norms must be established to serve as standards for measuring the degree of abnormality. This is probably nowhere more pressing than in connection with the hypophysis cerebri. The rapid changes in ideas regarding the significance of the different parts of the hypophysis, Geiling,[1] makes quantitative and more accurate statistical data especially urgent, for without this, erroneous deductions and undue speculation are apt to further complicate the situation.

Comparatively accurate figures on the variability of the main parts of the normal male adult hypophysis were recently published by Rasmussen.[2] It is, however, necessary to go further into the histology and cytology of this organ. The relative number of the various types of cells in the anterior lobe — whether they are different functional stages of the same cell or distinct types each with its own specific function, a question that is still debated — needs rather careful consideration on account of the many statements on variations from the normal that appear in the literature on pathology. A good example of this is a recent paper by Kiyono [3] on the histopathology of the hypophysis in which the author reports almost invariably either a decrease or an increase in one or more types of cell. In that particular study the most common change was an increase in the basophils. Next in order was an increase in chromophobes and still less frequently either an increase or a decrease in

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acidophils in about equal proportion. A decrease in basophils and chromophobes was noted only rarely. One wonders what criteria of normality were used.

There is a widespread notion that in the human adult acidophils predominate, basophils being next in number and chromophobes the fewest of all, Erdheim and Stumme,[4] Blair-Bell,[5] Erdheim,[6] Ewing.[7] Others agree that the acidophils are by far the most numerous and that the other types are present in relatively small quantities, but vary somewhat in regard to the relative proportions of the latter, Mott and Robertson,[8] Cooper.[9] But the most accurate statement seems to have been made nearly thirty years ago by Thom,[10] who estimated that at least a third are acidophils and that basophils are much fewer. This leaves the chromophobes as the most numerous.

Changes in the relative number of the different types of cells in old age are variously interpreted. Some think the chromophils decrease in advancing years, Erdheim,[11] Cooper.[9] Others claim that the basophils increase, Lucien,[12] Kraus.[13]

Of the many reported changes in the human hypophysis under physiological and pathological conditions (for an extensive bibliography see Erdheim[6]), an increase in acidophilic cells during pregnancy and the presence of acidophilic adenomas in acromegalia are the most thoroughly established. According to Bailey and Cushing[14] the acromegalic symptoms in dyspituitarism are roughly proportional to the presence of acidophilic granules in the tumor. Undoubtedly other important relations will be brought out when thorough examinations with improved technique have been carried out on a large series of hypophyses with known histories.

This investigation is an attempt to supply more accurate data on the variability in the proportions of the different cells in the anterior lobe of the hypophysis of supposedly normal male adults and to check some of the conclusions reviewed above. While as many as seven different kinds of cells have been described, only the three commonly accepted classes (chromophobe, acidophil and basophil) are clear-cut enough to be recognized with sufficient accuracy to admit of quantitative analysis. The chromophobes are also called chief cells, reserve cells, undifferentiated cells, and neutrophils. The acidophils are frequently termed eosinophils, oxyphils and more recently alpha cells. The basophils are also designated cyanophils

and beta cells. The relative merits of these terms need not be discussed here. It is but another example of lack of standardization in histological terminology and uniformity of technique.

METHOD

Histological Technique: The method has been explained in detail in an earlier paper, Rasmussen and Herrick.[15] In brief it consists of fixing in formalin and embedding in paraffin, orienting so that the hypophysis can be cut into serial sections in the horizontal plane. By determining the vertical diameter of the organ, the total number of sections can be calculated. After cutting about one fourth of the sections at 10 microns, ten sections at 5 microns are taken. Cutting is continued at 10 microns till halfway through the block, when ten more 5 micron sections are cut. When three fourths through, ten sections 5 microns thick are again taken. The rest of the block is cut at 10 microns.

Only the 5 micron sections are used for cell counting. The thick sections (if the organ is perfect) can be used for other studies. The thin sections from the three levels are stained by Mallory's acid fuchsin-orange G.-anilin blue method. In the later part of the work the nuclei were brought out more sharply by first staining about one-half to one minute in ordinary hematoxylin and washing well in distilled water before going into the acid fuchsin, as suggested by Brilmyer.[16] This facilitates counting. The essential staining technique, frequently termed Mallory's connective tissue stain, appears to be used more and more extensively for various purposes, either as originally used or with various changes. Some recent modifications have just appeared in a paper by Chiovenda.[17]

Several sections from each level are stained, varying the time in the different solutions as trial staining indicates. On reasonably fresh material fixed in formalin or corrosive sublimate, or both, the three kinds of cells stand out in great contrast.

Counting: The most favorable section from each level is explored systematically under an oil immersion lens on a binocular microscope by means of a mechanical stage. The epithelial cells in every fifth microscopic field in every fifth row of fields are counted. Only cells containing a nucleus are considered, in order not to overevaluate the larger cells. A large cell is cut into more sections than

a small cell and hence appears more numerous than it really is. The nuclei of the different types of cells average nearly the same in diameter.

Each field is naturally subdivided into irregular areas by blood channels and strands of connective tissue, so that it is fairly easy to keep in mind the parts of the field that have been traversed in the process of counting. Even in 5 micron sections it is necessary to focus up and down on every group of cells in order to bring into view all the nuclei within the section.

In this way there are obtained from about 100 to 350 microscopic fields and a total cell count of about 10,000 to 30,000 cells in each hypophysis. The results do not differ more than 2 per cent when compared with a count on sections from 20 to 30 levels, which naturally requires much more time.

Plane of Section and Pattern of Cell Distribution: If sagittal sections were used many more levels would have to be used because of the irregular distribution of the various kinds of cells. The pattern of arrangement is much more uniform in horizontal series. The acidophils are more or less concentrated into two areas which occupy much of the central and posterior part of each lateral half of the lobe. In the midsagittal plane and anterior and lateral margin, frequently extending back to the posterior lobe, the acidophils are less numerous and hence the chromophobes and especially the basophils are more numerous than elsewhere.

Selection of Material: In order to get as normal material as practicable, only specimens with no evident pathology, from cases of sudden and usually accidental death, were used, and then usually only if fixation occurred within twelve hours after death. A few hypophyses that seemed unusually well preserved after a somewhat longer postmortem period are included.

Results

Frequency Distribution: The distribution of the percentages is shown in Charts 1, 2 and 3. These graphs are fairly symmetrical for this kind of data. The chromophobes are skewed slightly in the negative direction, while the acidophils and basophils show slight positive skewness. We have no explanation for the bimodal distribution of the chromopholes.

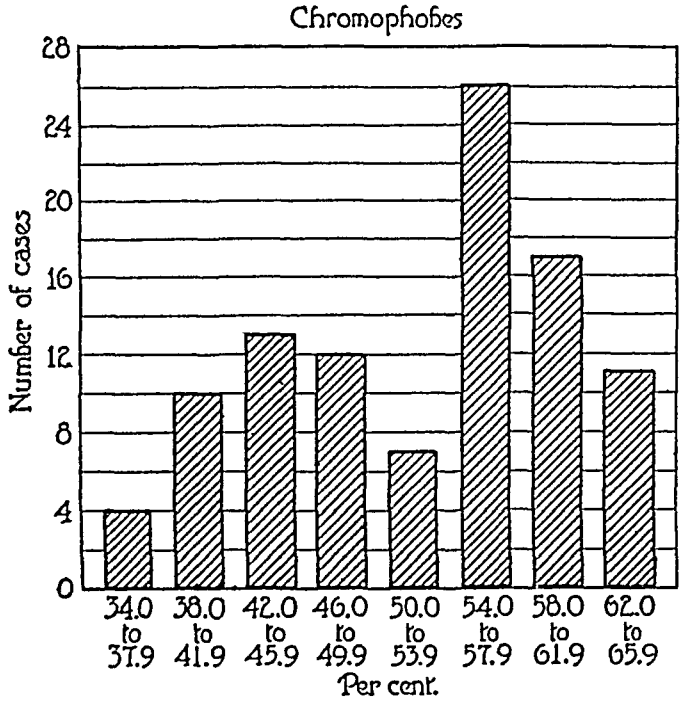


CHART 1. Frequency distribution of the percentage of the chromophobes in the anterior lobe of the normal male adult hypophysis.

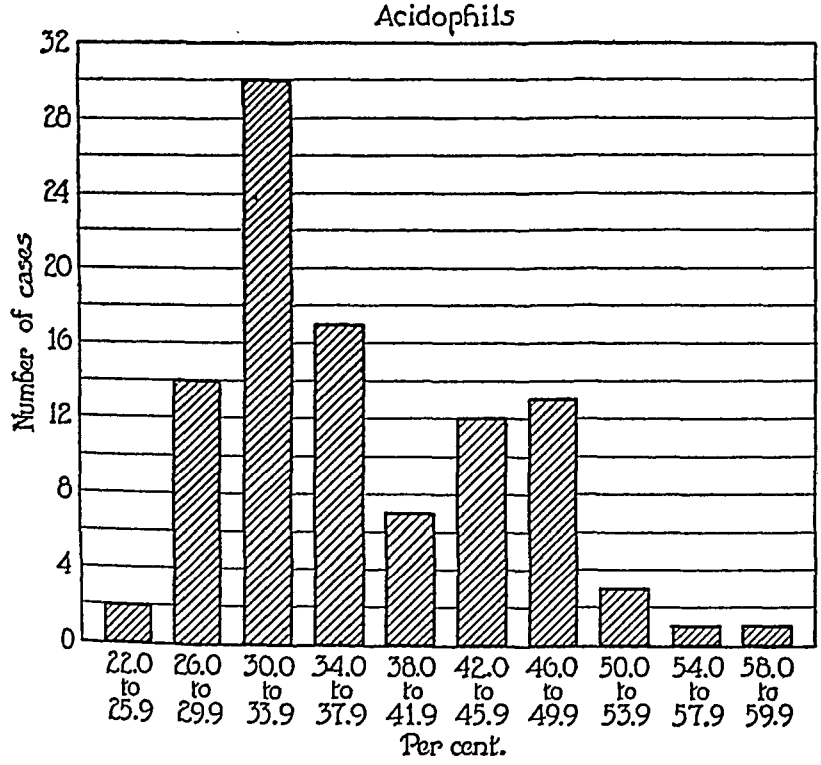


CHART 2. Frequency distribution of the percentage of the acidophils in the anterior lobe of the normal male adult hypophysis.

Percentage of Chromophobes: As shown in Table I, the chromophobes vary from 34 per cent to 66 per cent, the central tendency being somewhat over 50 per cent. The great scarcity of chromophobes so often mentioned is not substantiated. In no single case

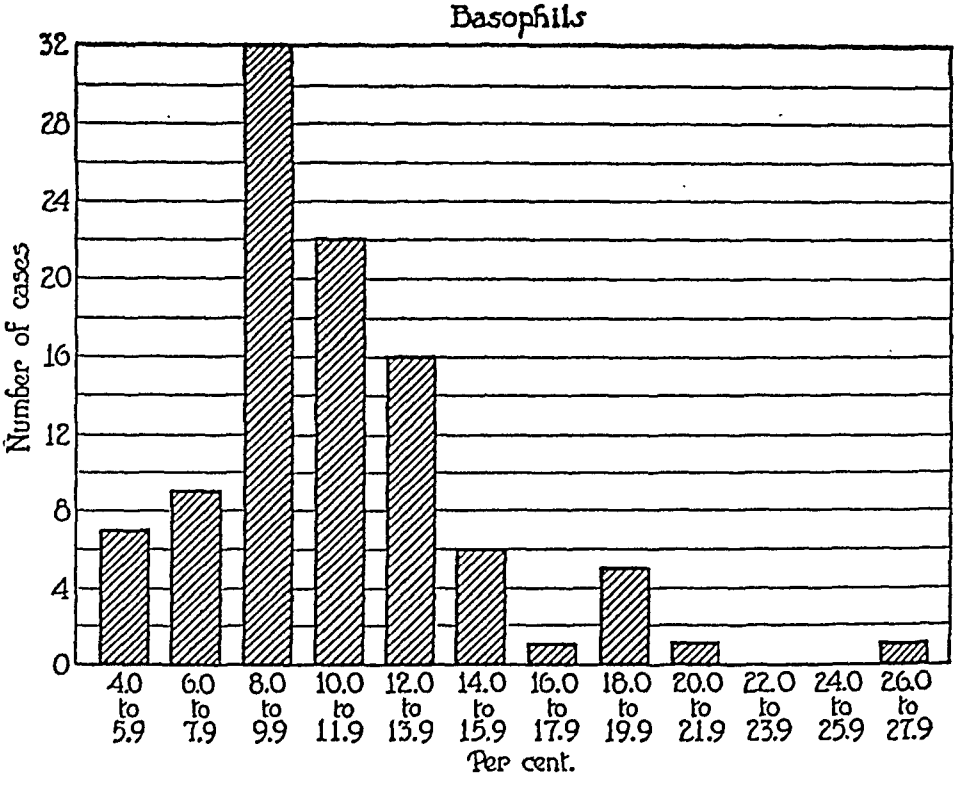


CHART 3. Frequency distribution of the percentage of the basophils in the anterior lobe of the normal male adult hypophysis.

did they constitute less than one third of the total cells. It must be remembered that many chromophobes contain practically no cytoplasm and hence are in the background of the picture and generally underevaluated. The average is 52.2 per cent. The coefficient of variability is 15. This is the same degree of variation as in the weight of the liver and slightly less than the variability of the kidney and heart whose coefficients of variability are respectively 17 and 18.

Percentage of Acidophils: The acidophils (Table I) are less numerous, varying from about 23 per cent to 59 per cent, and having a mean value of 36.8 per cent. The coefficient of variation is 21. This is the same degree of variability as is found in the weight of the anterior lobe of the hypophysis. In only five cases did the acidophils

constitute one-half or more of the total number of cells. The tendency of so many investigators to regard these cells as being the most numerous is very likely because they are very conspicuous, due to their size and intense staining reaction.

Percentage of Basophils: The basophils (Table I) are the least numerous, representing only 11 per cent. They are also the most variable in number, ranging from 4.5 per cent to over 27 per cent. The coefficient of variation is 34. This is about the variability of the weight of the normal spleen and thymus.

TABLE I

Percentage of the Different Types of Cells in Anterior Lobe of Human Hypophysis (100 Normal, Male, Adults)

Cell types	Minimum	Maximum	Median	Mean and probable error	Standard deviation	Coefficient of variation
Chromophobe	34.0	65.9	54.3	52.2 ± 0.54	7.98	15
Acidophil.....	22.6	58.9	34.8	36.8 ± 0.52	7.78	21
Basophil.....	4.5	27.4	10.0	10.9 ± 0.25	3.71	34

Relation to Body Weight: The body weight given in the autopsy records is, as a rule, only a rough estimate, so that a careful study of the relation of body weight to the percentage of the relative number of the different cells in the anterior lobe of the hypophysis cannot be made; but it seemed worth while to compare a number of unusually heavy persons with those of more ordinary weight. Several of these over-weight cases had the general external features of so-called non-neoplastic postadolescent hypopituitarism, although the hypophysis was apparently normal in every respect. The results are found in Table II. The fourteen individuals over 200 lbs. in weight had on an average 3.5 per cent more chromophobes and 4.6 per cent less acidophils than the eighty-six individuals under 200 lbs. in weight. The statistical significance of these differences is doubtful because the ratio of the differences to the probable error of the difference (see last column of Table II) in neither case is 3 or more. It is probable, however, that there is a slightly lower percentage of acidophils in extra heavy people.

Relation to Stature: It has been well established that there is a positive correlation between the weight of the entire hypophysis and body length, Rasmussen.[18] This has been found to be due to a

larger anterior lobe in taller individuals.[2] Since apparently it is the acidophils that are associated with growth, it is worth while to see if there is any correlation between the relative number of the different types of cells and height. In the cases here involved the length of the body is recorded to within a half centimeter. Table III shows that there is essentially no difference in the percentages be-

TABLE II

Mean Percentage of the Different Types of Cells in the Anterior Lobe of the Hypophysis in 14 Cases 200 to 330 lbs. in Body Weight Compared with 86 Cases Under 200 lbs. in Body Weight

Cell types	200–330 lbs. (14 cases)	Under 200 lbs. (86 cases)	Difference	P.E. of Diff.	Difference / P. E. of Diff.
Chromophobe	55.2 ± 1.47	51.7 ± 0.56	+ 3.5	1.57	2.2
Acidophil.....	32.9 ± 1.50	37.5 ± 0.55	− 4.6	1.60	2.9
Basophil.....	11.9 ± 0.63	10.7 ± 0.27	+ 1.2	0.69	1.7

tween persons above average height and those below. This is also borne out by the low and statistically insignificant coefficients of correlation (r) listed in the middle column of Table V.

Relation to Age: Because of the fact that the anterior lobe decreases noticeably in size after 50 years of age,[2] the thirty-one cases over 50 years old were compared with the sixty-nine cases under that

TABLE III

Mean Percentage of the Different Types of Cells in the Anterior Lobe of the Hypophysis in 52 Cases Above Average Stature Compared with 47 Cases Below Average Stature. Mean Stature = 172.5 cm.

Cell types	Above average (52 cases)	Below average (47 cases)	Difference
Chromophobe.....	52.6 ± 0.77	51.6 ± 0.75	+ 1.0
Acidophil.....	36.5 ± 0.76	37.5 ± 0.72	− 1.0
Basophil.....	10.8 ± 0.34	10.9 ± 0.37	− 0.1

age. The differences between the average percentages and a test of the significance of the differences are recorded in Table IV. The prevalent idea that the basophils increase with age in adults is not supported.

If the basophilic cells which invade the posterior lobe were included, there would undoubtedly be a noticeable increase, because

those cells are largely, if not entirely, responsible for the gradual increase in the total volume of pars intermedia with age, Rasmussen.[19] While there are those (especially Kasche [20] and Dayton [21]) who regard the basophilic cells in the pars nervosa as having originated from the anterior lobe or differentiated into anterior lobe cells, there is considerable evidence that they arise from the posterior wall of Rathke's pouch and hence should be considered as part of pars in-

TABLE IV

Mean Percentage of the Different Types of Cells in the Anterior Lobe of the Hypophysis and Age

Cell types	51–78 yr. (31 cases)	18–50 yr. (69 cases)	Difference	P. E. of Diff.	Difference / P. E. of Diff.
Chromophobe	54.8 ± 0.91	51.1 ± 0.56	+ 3.7	1.07	3.5
Acidophil	34.2 ± 0.86	38.1 ± 0.63	− 3.9	1.07	3.6
Basophil	11.0 ± 0.43	10.8 ± 0.31	+ 0.2	0.53	0.4

termedia, Guizzetti,[22] Lewis and Lee.[23] On the assumption that they belong to pars intermedia, cells posterior to the cleft, or residual lumen, have not been included in this enumeration.

In the case of the percentage of chromophobes, however, there is a distinct increase in the older group and a corresponding decrease in the percentage of acidophils, amounting to nearly 4 per cent. The ratios of these differences to the probable error of the difference (see last column of Table IV) are 3.5 and 3.6, indicating that

TABLE V

Coefficient of Correlation (r) between Percentage of Cells and Stature and Age

Cell types	Stature	Age
Chromophobe	+ 0.152 ± 0.066	+ 0.199 ± 0.065
Acidophil	− 0.168 ± 0.065	− 0.221 ± 0.055
Basophil	+ 0.035 ± 0.067	− 0.022 ± 0.065

the change with age is fairly certain. This is supported also by the coefficient of correlation (r) listed in the last column of Table V. A decrease in acidophils in advancing years is the least questionable because the ratio of the probable error to the coefficient of correlation is at least 4.

Summary

1. The necessity of a more accurate estimation of the relative number of the different types of cells in the anterior lobe of the human hypophysis is evident from the great divergence of opinion that exists among even recent writers in regard to what should be considered normal proportions and from the greater attention being paid to the pathology of this organ.

2. The results of making a differential count of the chromophobes, acidophils and basophils in 100 selected and supposedly normal male adult hypophyses from cases of sudden accidental death, in which the tissue in general was fixed within twelve hours after death, are presented.

3. The data are based on a differential count of from 10,000 to 30,000 cells from 100 to 350 microscopic fields taken systematically from three different regions of a complete series of horizontal sections from each hypophysis.

4. The chromophobes represent on an average 52 per cent of the total cells. They vary from 34 per cent to 66 per cent. The coefficient of variation is 15.

5. The acidophils average 37 per cent, varying from 23 per cent to 59 per cent, the coefficient of variation being 21.

6. The basophils strictly within the anterior lobe are the least numerous and the most variable. The average is 11 per cent, but they range from less than 5 per cent to more than 27 per cent. The coefficient of variation is 34.

7. Fourteen cases with a body weight 200 lbs. to 330 lbs. averaged 3.5 per cent more chromophobes and 4.6 per cent less acidophils when compared with eighty-six cases under 200 lbs. in weight. Statistically this difference is not enough to be taken seriously, although the decrease in acidophils is probably real.

8. There is no correlation of importance between body length and percentage of any particular type of cell.

9. Individuals over 50 years of age average nearly 4 per cent more chromophobes and correspondingly less acidophils.

10. There is no correlation between age and basophils in the anterior lobe of the hypophysis.

NOTE: Just as this paper went to press, there appeared a report by J. L. McCartney (Dementia precox as an endocrinopathy with clinical and autopsy reports. *Endocrinology*, 1929, xiii, 73) which seems to be one of the first at-

tempts to determine the percentage of the different cells in the anterior lobe of the hypophysis upon a large scale. In 158 male cases of dementia precox he found an average of 40 per cent neutrophils, 43 per cent eosinophils and 17 per cent basophils. In 241 males with other forms of psychosis the percentages are: 20 per cent neutrophils, 52 per cent eosinophils, 28 per cent basophils. In 24 dementia precox females the percentages are: 27 per cent neutrophils, 52 per cent eosinophils, 21 per cent basophils. In 64 females with other forms of psychosis, the corresponding figures are: 23 per cent neutrophils, 57 per cent eosinophils, 20 per cent basophils. While no information is given as to technique or criteria used, the low neutrophil count suggests a wide departure from the chromophobe count in normal cases or more likely a very restricted definition of the neutrophilic group, such as to exclude those with little or no cytoplasm. This shows the importance of some standard that will be generally acceptable.

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A MALIGNANT MEDIASTINAL TERATOMA *

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HISTORY †

The patient was a white male, 27 years of age, by occupation a locomotive fireman. Three months prior to his death he was engaged in firing a locomotive on a run, when he slipped on the floor of the cab, falling against the fire box with his left shoulder. He continued his work to the end of the day. Within twenty-four hours he was seen by a physician who found no external signs of injury, but advised him to rest for a few days and then, if able, to resume work. After he returned to work he began to complain of a continuous pain in his left shoulder and chest, with some difficulty in breathing. Then there followed a period of about eight weeks during which there were no observations and at the end of which, because of increasing disability, he was admitted to the hospital. In the previous history it is stated that the patient had lobar pnuemonia nine years ago. The only important findings on physical examination were signs suggestive of infiltration in the upper lobe of the left lung.

Shortly after admission to the hospital signs of meningeal irritation developed, with cervical rigidity and a positive Kernig's sign. A tap of the spinal fluid showed it to be clear, not under pressure; on examination increased globulin was found and a cell count of 30 cells; on standing a definite web formed. A tentative diagnosis of pulmonary tuberculosis with complicating tuberculous meningitis was made at this time.

The patient died under symptoms of rapidly increasing dyspnoea and cyanosis some fourteen weeks after the date of the alleged accident, and three weeks after he entered the hospital. The temperature during his stay in the hospital ranged from 100° to 101° F. The postmortem examination was made within three hours after death.

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Necropsy

The body is warm, rigor mortis absent. Nutrition poor; length, 169 cm.; estimated weight, 59 Kg. Pulmonary arthropathy, absent.

On removal of the sternal breast plate a large tumor mass is found presenting in the median line above the heart, extending up on the great vessels to the suprasternal notch. It is adherent to the right lung and the pericardium and measures 14 cm. in the vertical diameter, 8 cm. laterally, and 9 cm. anteroposteriorly. On palpation it conveys a sense of fluctuation. On section it is found to be a thick-walled cyst, measuring 2 by 3 mm. at the lower and 3 cm. at the upper pole, with a nodule presenting on the inner surface above, from which delicate hairs seem to be growing. The cavity is filled with sebaceous material, mixed with hair.

On further dissection the tumor is seen to embrace the superior vena cava and to be compressing it; the intima of that vessel while smooth, exhibits slight nodular bulgings. The course of the aorta, however, is not compromised, and the trachea and bronchi show no compression although they are in relation with the tumor anteriorly. The upper and lower tracheobronchial and paratracheal lymph nodes are all merged into the tumor mass, being variously enlarged, averaging 1 to 2 cm. in diameter and showing on section a diffuse scirrhous tissue. The dissection of the cervical region reveals a large node 2.5 cm. by 2 cm. at the lower pole of the left thyroid lobe and a somewhat smaller one at the upper pole, the larger showing several circumscribed grayish nodules, the other suggesting caseation.

The entire right lung is adherent. Both lungs crepitate well throughout and on section a considerable quantity of thin blood-stained froth exudes from the cut surface.

In the heart there are no remarkable changes excepting a slight hypertrophy of the right ventricle.

On removal of the calvarium the veins and sinuses appear engorged; the cerebrospinal fluid is slightly increased, the convolutions flattened to a moderate degree, the meninges over the base and tips of the temporal lobes, and in the extensions into the Sylvian fissure are thickened and opaque, with numerous fine nodules not unlike tubercles in appearance. The ependymal lining of the ventricles is smooth, the choroid plexus, pituitary and pineal bodies are not remarkable. The accessory sinuses and both middle ears are free from changes.

The abdominal organs are all normal in size, appearance, position, and in their relations to each other, excepting a considerable hyperemia which is general in its distribution.

The kidneys, adrenals, urinary bladder, prostate, seminal vesicles, and testes show nothing remarkable.

Microscopic Examination

All the lymph nodes examined exhibit an adenocarcinomatous invasion, completely replacing the lymphadenoid tissue. The cells of the tumor show considerable variability in arrangement, though a tendency to alveolar formation is constant. In shape they are flattened or polyhedral, and there are numerous mitotic figures. This form of tumor is also found in the thyroid. Sections from parts of the lungs which grossly appeared free from tumor show metastases in the form of peribronchial and perivascular infiltration and filling of lymphatic vessels with tumor cells. In one section of the lung the tumor was found to have invaded the wall of a pulmonary vein, with tumor cells mixed with the red corpuscles inside the lumen of the vessel. Infiltration of tumor cells arranged in irregular strands and groups in close relation to the vessels, with some few collections of small round cells, can also be seen in the meninges.

The various sections from the main tumor mass show complex pictures including islands of well formed hyaline cartilage, strands of striated muscle, smooth muscle fibers, dense hyalinized connective tissue, with cystic spaces lined with endothelium, an island of nerve fibers, an epidermis-like structure in cyst formation, and an area containing hair follicles and islands of sudoriferous glands, all quite mature and readily identified; also numerous larger irregular cystic cavities lined with ciliated epithelium and large areas of necrosis containing calcium deposits; finally structures resembling osteoid tissue.

Discussion

The tumor presented in the foregoing may be described as a partly solid and partly cystic tumor of the mediastinum composed of representatives from all three layers of the blastoderm in mature form, therefore a mediastinal teratoma. A malignant transformation took place within this tumor in the form of an adenocarcinoma. There

was direct extension of the growth into the lungs and metastasis into the cervical lymph nodes, thyroid and meninges.

As to the literature on the subject of mediastinal tumor there are several good reviews, so that only a brief resumé touching the principal points of interest will be given here. The first case was published by Gordon [1] in 1823. The tumor was situated in the anterior mediastinum well up under the sternoclavicular junction. It was cystic and contained the usual sebaceous material with hair; also seven teeth and some bone. In the succeeding years, a considerable number of isolated cases were reported and toward the end of the century these were followed by a notable series of papers which, besides adding to the number, included comprehensive reviews. Among these are the papers of: Hoffman [2] (1896) covering twelve cases; Pflanz [3] (1896) including twenty-four cases; Christian [4] (1902), forty cases; Dangschat [5] (1903), forty-four cases; Hertzler [6] (1916), seventy-two cases; Kleinschmidt [7] (1920), seventy-two cases; Lambert and Knox [8] (1920), seventy-nine cases; Terplan [9] (1922), sixty-eight cases. A search of the literature since 1920 yields twelve more cases. These added to those included in the above-mentioned reviews, make with Gordon's first case, in a period approximating 100 years, about a hundred cases. However, comparatively few of these exhibited a malignant phase. Lambert and Knox estimated 11 per cent of malignancy in seventy-nine cases. The tumors are generally found to be cystic with a distinct solid portion, but occasionally are entirely solid. The malignant feature has been found associated with true teratomas both cystic and solid.

As regards the age incidence, these tumors have been found in both extremes of life, some in individuals as young as 2, 4 and 6 years, and some in individuals 50 to 70 years of age. The vast majority, however, are confined to early adult life (20 to 30 years). Pflanz regarded the puberty period as particularly stirring the latency of these tumors into activity, likening it to the awakening of sex characteristics, especially hair distribution and beard growths. In sex incidence these tumors would appear to be equally divided. Trauma has frequently called attention to their presence.

The signs and symptoms of this type of tumor in the mediastinum have been confused with those of tuberculosis since earliest times and a number of cases have been reported where pulmonary tuberculosis coexisted. Thoracic aortic aneurism and ecchinococcus cyst

have also been mistaken for these tumors. The diagnosis has been at times made by operative procedure such as a trocar puncture, with the typical yield of sebaceous material, epithelial débris, cholesterin and hair. Erosion into a bronchus has been reported a number of times with the raising of the characteristic cyst content in the sputum. Pulmonary arthropathy has been reported in a number of cases. Roentgenologic examination in recent years has aided in making the diagnosis in several instances. However, in the majority of cases the tumor was discovered by postmortem examination. The duration of the symptoms has been found to range from six weeks to two and three years with the final issue always fatal. The more common locations are found to be in the anterior mediastinum, usually to one or the other side of the median line, above the bronchial tree, embracing more or less the great vessels, trachea and bronchi, in intimate relation with the base of the heart, pericardium and mediastinal pleura, compressing one or the other lung to a varying degree. In size these tumors range from three to four centimeters in diameter all the way to the size of a child's head. The average is about fifteen centimeters for vertical diameter and six to seven for lateral and anteroposterior diameter. Some few have been reported in the posterior mediastinum and some extending below the bronchial tree, resting on the diaphragm.

It is now generally accepted that the "anlage" for these tumors is in the cervical region during the early embryonic period along with the lungs, heart, and thymus, before the closure of the blastodermic folds and that they move into the thorax with these organs. This is suggested rather strikingly in a number of cases, notably that of Collenburg [10] whose case was found to have a band-like connection, that extended from the posterior part of a large tumor about the size of a child's head, in the right lower anterior mediastinum, upward to the lower edge of the right lobe of the thyroid. In this band of tissue he was able to demonstrate a branch of the inferior thyroid artery, also two veins that he could trace into the inferior thyroid vein, with muscle fibers from the right sternothyroid muscle. Collenburg was thus the first to suggest the cervical anlage of the mediastinal dermoid, he also assumed that it was the thymus that gave rise to it. Pinder's [11] case also showed a band of connective tissue that extended from the posterior part of this tumor up to the suprasternal notch. From this connection and position Pinder as-

sumed that the tumor originated with the thymus. Marchand [12] also took the position that in time of origin these tumors must be classed with the early position and time origin of the thymus. This view was endorsed with more or less qualification by others who followed, notably, Christian, Dangschat, and Hertzler.

While there appears to be agreement to a certain extent regarding the time origin and the evolution of position, the histogenesis is still subject to conflicting theories. The earliest theory was that these tumors were the result of the constriction of an invaginated portion of the ectoderm which became included on closure of the blastodermic folds. Attention was called to the fact that tumors having entodermic elements (Dangschat) could not thus be accounted for unless it was assumed that at the time a portion of the entoderm was included, and the theory failed entirely when considering the more complex types. The thymic origin was the next theory, in chronological order, advanced for these tumors, the proponents arguing that since the anlage for the thymus was in the third pharyngeal pouch, the thymus therefore was endowed with both meso- and entodermic properties, that it could therefore account for two blastodermic representatives. Virchow [13] suggested that the thymus or branchial cleft should be considered as potential sources of origin for mediastinal tumors of this type. Marchand, Pinder, Collenburg, and Ritchie,[14] each accepted this theory in the order named. The thyroid, too, came under suspicion in the same way because of its origin in the fourth pharyngeal pouch, but both of these theories were found to be wanting when the nervous tissue elements and organ rudiments, as well as other mature tissue elements, were to be accounted for. Certainly their application would fit only a limited number of cases.

The branchial cleft theory suffered in the same way, although supported for a time by Virchow, Lindstedt,[15] Pflanz, Marchand, and Christian. Lindstedt, who reported a case of mediastinal tumor that he characterized as an adenomyxochondrosarcocarcinoma, assumed that it was bronchogenic in origin, basing this assumption upon the arrangement of cartilage, smooth muscle, and ciliated epithelium that suggested the respiratory tract. He admitted, however, the presence of connective tissue in all forms, adipose tissue, islands of well formed cartilage, numerous cavernous blood spaces, and epithelial elements in glandular, alveolar and cystic arrange-

ment. It seems therefore that his case surely should be classed with the more definite teratoid tumors.

"Inclusio foetus in foetu" as a theory was introduced early as more nearly accounting for the vast majority of these mediastinal tumors. Ekehorn [16] was the first to take this advanced position, basing it on the case that he reported in which he found in addition to cartilage and bone a portion of bone in which he saw resemblance to a superior maxilla with a number of teeth in it, definite incisors being recognized. In addition he found nerve fibers, ganglion cells and numerous canals that in their arrangement suggested respiratory tract, and portions in which he saw a resemblance to intestine. Dangschat also held that this theory more clearly met the requirements of this anomaly. Askanazy [17] saw in it an analogy to partial fruition of a second pregnancy. This bigerminal theory still persists in the minds of many of the later observers. Finally the isolation of a blastomere, the delayed division of such blastomere, and the possible fertilization of a polar body, were advanced toward solving this question.

The publication of Budde [18] presents a good review of these later theories. He concluded that all tumors that do not exhibit elements traceable to each of the three layers of the blastoderm, are in the nature of definite "local defects." They are tumors that have their origin at a later period of the embryo, at a time when organs and organ systems are in their early stages of budding, suggesting that at this time invaginations and foldings might well isolate a cell complex from which such tumors could arise. He classifies them as "hamartomes," among which he includes the true dermoids, branchial cleft tumors and those arising from thyroglossal duct remnant. The finding of nervous tissue elements he regards as definite criteria for differentiating hamartoma from teratoma. He further asserts that the undoubted bigerminal twin parasite should always be found free and never enclosed within the body cavity, and further, that it should always exhibit traces of vertebral segment to prove its metamere structure.

The following is a list of all the types of malignancy that have occurred in this group of tumors.

1. Virchow's case showed both carcinomatous and sarcomatous nodules, that had metastasized in the liver.

2. Ceelen's [19] case showed irregular adenocarcinoma with many mitotic figures in a cystic mediastinal dermoid with invasion of the lung, and lymph node metastasis.

3. Jores,[20] described his case as a cystosarcoma with pulmonary metastasis.

4. Pinder, lymphosarcoma in part spindle-celled.

5. Ritchie, embryoma in mediastinum.

6. Lindstedt, adenomyxochondrosarcocarcinoma.

7. Lambert and Knox, chorionepithelioma.

8. Warthin (cited by Lambert and Knox), chorionepithelioma.

9. Pohl,[21] a child three years of age that showed a mediastinal tumor with sarcomatous transformation.

10. Schutt (cited by Lambert and Knox), an alveolar sarcoma and adenocarcinoma both of which were found to have invaded blood vessels.

11. Black (cited by Lambert and Knox), some few carcinomatous areas.

12. The case reported above, adenocarcinoma.

Summary

1. A case of malignant teratoma is reported.

2. A resumé of the literature indicates that these tumors should be classed as teratomas rather than as dermoids.

3. About 10 per cent of these tumors undergo sarcomatous or carcinomatous transformation.

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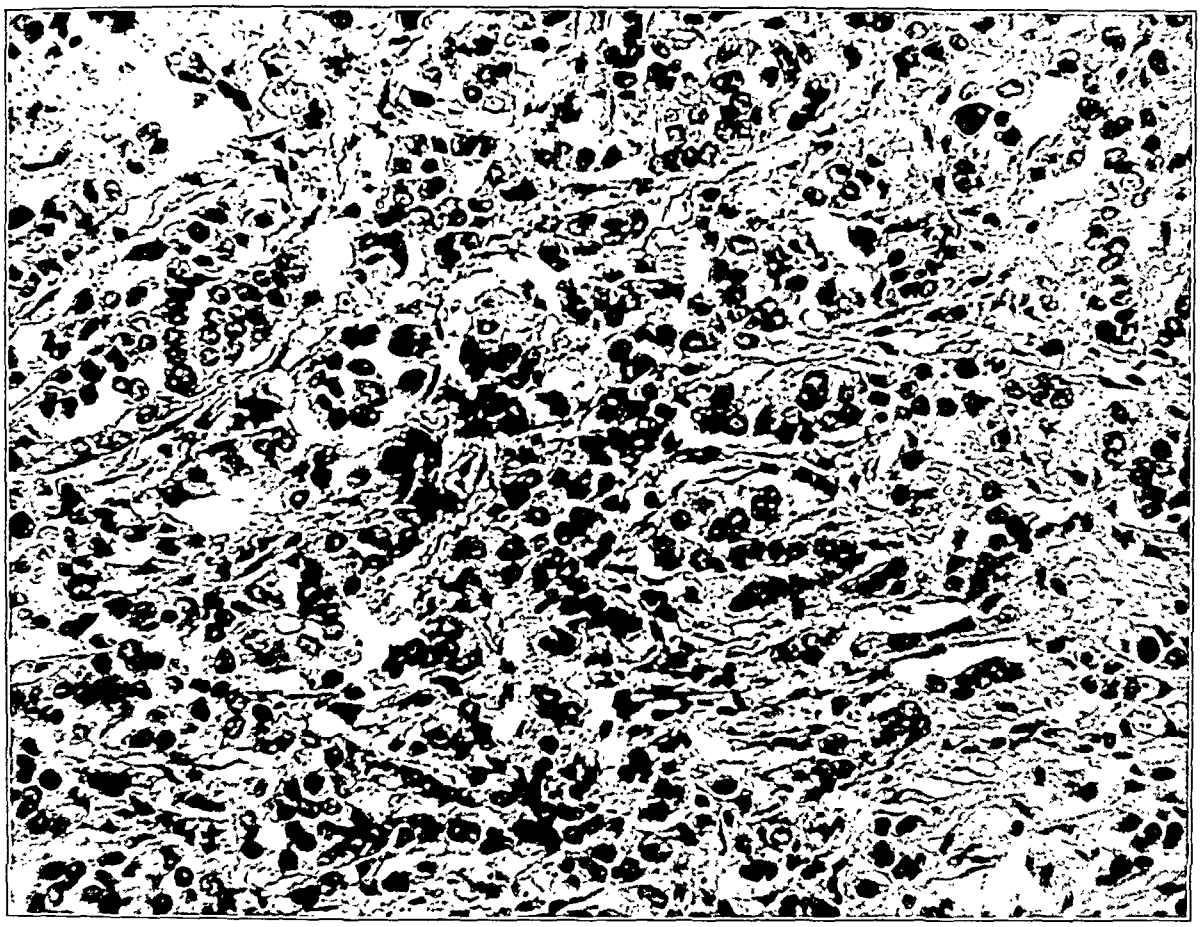
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DESCRIPTION OF PLATES

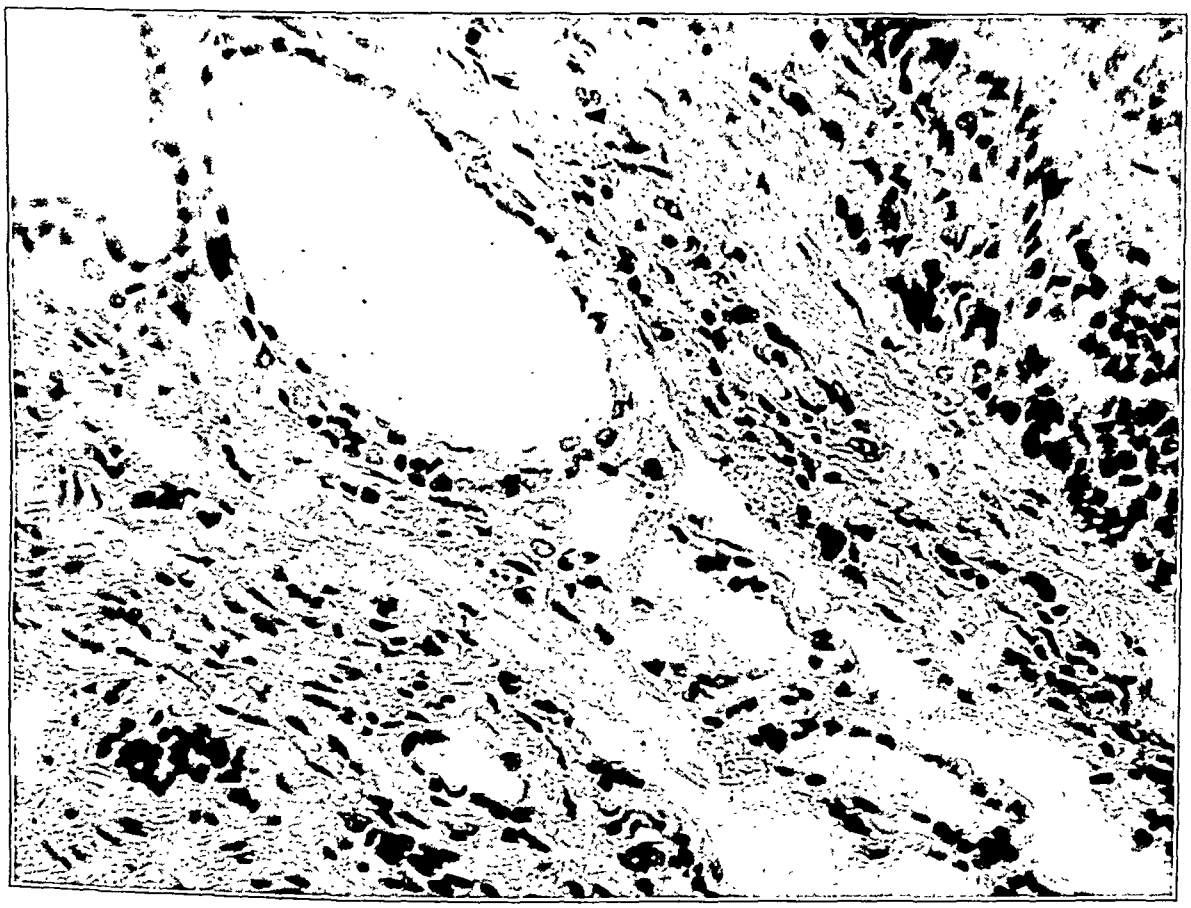
PLATE 55.

FIG. 1. A section from the main tumor showing the adenomatous character.

FIG. 2. Invasion of thyroid.



1



2

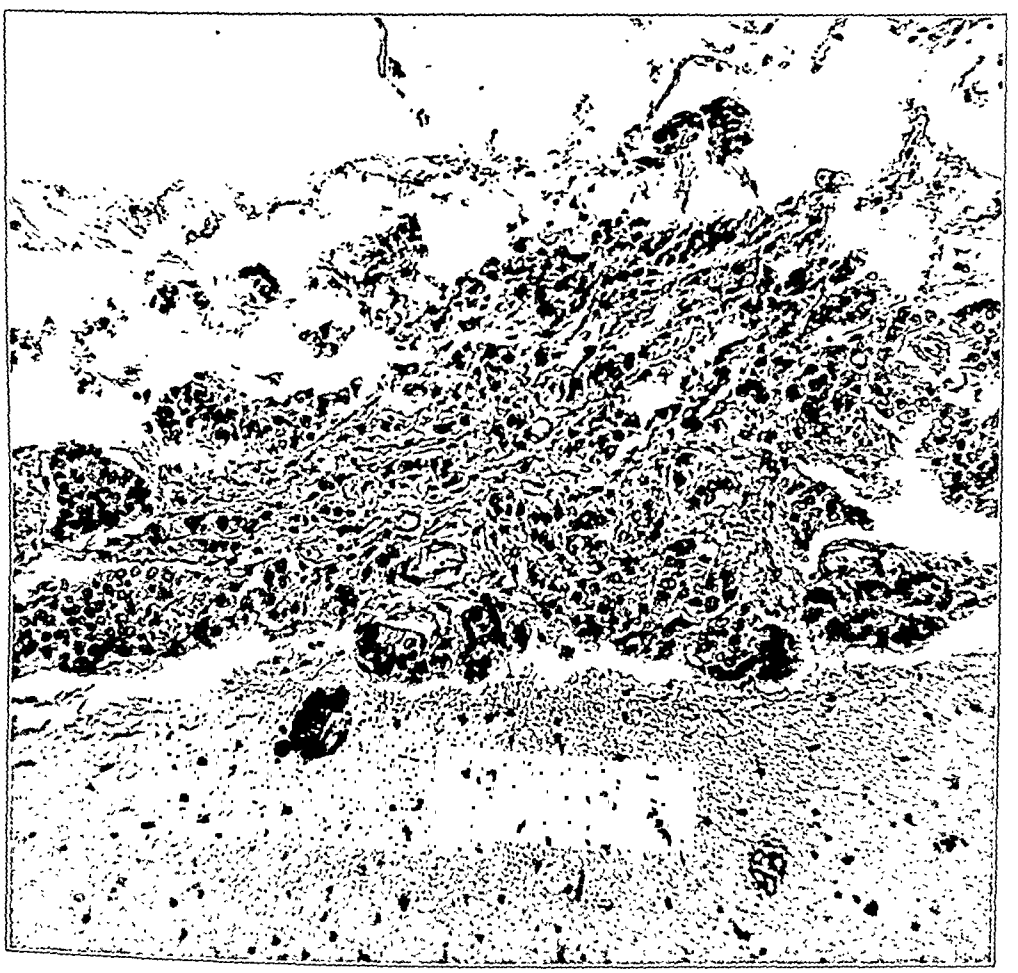
PLATE 56.

FIG. 3. A posterior view of tumor showing its relation to trachea and bronchial tree.

FIG. 4. Tumor proliferation in the meninges.



3



4

Jacobs

Malignant Mediastinal Teratoma

TWO OF THE RARER CONGENITAL ANOMALIES OF THE HEART*

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I. ABSENCE OF ONE PORTION OF THE AORTIC ARCH WITH STENOSIS OF THE AORTA AND WITH DEFECT IN THE INTERVENTRICULAR SEPTUM

Stenosis of the isthmic part of the aorta is quite common but besides this developmental failure other anomalies may occur in this region. Such developmental anomalies are: (1) destruction of the isthmus in which there is a thicker or thinner connective tissue bundle present between the arch and descending portion of the aorta; and, very rarely, (2) an entire absence of this portion. The absence of this isthmus is the most extreme grade of malformation occurring here. It is characterized by the finding that the aortic arch and descending aorta end blindly and that there is absolutely no connection between them. About eight such cases are described in the literature. The blood of the right ventricle empties into the thoracic part of the descending aorta, but there are usually other anomalies present which cause the venous blood to be mixed in the heart, and in such cases life is prolonged.

Not only the isthmus of the aorta but a larger portion of the aortic arch may be absent. This condition occurred in the following case and was associated with other cardiac anomalies.

CASE I

Clinical History: A small undernourished, white, female infant of 6 months draining profusely from both ears. She has vomited continually since birth. The heart is enlarged and there is a loud, blowing murmur heard over entire precordium. No thrills are felt. The child becomes very cyanotic during crying spells. It was admitted with a diagnosis of bilateral otitis media and congenital heart disease.

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Necropsy Report: (No. A. 28–80). The postmortem examination reveals a bilateral paravertebral type of bronchopneumonia as the cause of death. A chronic catarrhal colitis was also found involving the transverse and descending colon and the sigmoid.

The position of the enlarged heart is normal. Its measurements are: length 60 mm., transverse diameter 62 mm., sagittal diameter 37 mm. The left ventricle forms the apex but the right ventricle is much larger than the left. The pericardium and endocardium are smooth. The auricles are normal in size and position, and the venae cavae and pulmonary veins empty in the proper auricles. The foramen ovale is patent, and is 2 by 4 mm. in size. Both ventricles are dilated, and their muscular walls are hypertrophied. The wall of the right ventricle is 1 cm. and the wall of the left ventricle is 7 mm. in thickness. The mitral and tricuspid valves are in their normal anatomical position and are smooth and thin without evidence of inflammation. The pulmonary artery is wide, 15 mm. in diameter, and its semilunar valves are large but thin. The wide opened ductus arteriosus empties into the descending thoracic aorta at which junction the left subclavian artery is given off. The aortic opening is diminished in size so that its diameter is only 5 mm. The three aortic valves are small, corresponding with the narrowed lumen, but they are normal. Right and left coronary arteries originate from the corresponding valvular sac. The much narrowed aorta branches into the innominate and left common carotid arteries but a portion of the arch between this and the left subclavian artery is entirely missing. Even a single, string-like connective tissue strand is absent (Fig. 1). There is a round defect, 8 mm. in diameter, in the interventricular septum under the orifice of the large arteries. Both ventricles are connected through this defect. It is lodged in the posterior half of the interventricular septum (Fig. 2).

Summarizing the pathological changes of the heart, we find: (1) an entire absence of one part of the aortic arch; (2) a patent ductus arteriosus; (3) a patent foramen ovale; (4) a very much narrowed aorta; and (5) subaortal defect in the interventricular septum.

Discussion

The most interesting and important anomaly in this case is the missing portion of the aortic arch. To understand better the existence of such a possibility, we must recall the developmental procedure of the vessels. At about the third week of embryonic life, six lateral branchial vessels arise from the divided truncus arteriosus (Fig. 3*a*). In the course of development modifications occur which consist in the disappearance of several arches. In this way the first, second and fifth pairs of arches and the right sixth arch disappear. From the other arches the permanent arteries are formed. The third pair becomes the external carotid arteries. The right fourth arch forms the proximal portion of the right subclavian artery. The left fourth branchial vessel together with the left dorsal longitudinal stem become the arch of the aorta. The sixth branchial vessels become the pulmonary arteries, the persisting connection of the left one being the ductus arteriosus (Fig. 3*b*). In our case, this normal development did not occur. The portion of the aortic arch lying between the left common carotid artery and ductus arteriosus is missing because the left fourth branchial arch was not laid down in embryonic life. If the fourth branchial arch had been present in early life, we would find a very thin, fibrous bundle connecting the ascending and descending aorta. Only the pulmonary artery is connected with the descending thoracic aorta by the dilated ductus arteriosus (Fig. 3*c*). Accordingly, the descending aorta arises from the pulmonary artery.

Another developmental anomaly of the heart was the stenosis of the aorta. Aortic stenosis is rarer than stenosis of the pulmonary artery. In this case the position of the aorta and pulmonary artery was normal, the aorta arising from the left ventricle posteriorly on the left and the pulmonary artery anteriorly on the right. The truncus septum divided the truncus arteriosus into two unequal parts, but it was rotated 225 degrees as it is usually, and its plane corresponded with the interventricular septum. The interventricular subaortic septum defects are usually due to the imperfect rotation of the septum trunci. The membranous septum is finally closed by the septum trunci if its plane is the same as that of the interventricular septum. Otherwise the fusion fails and various-sized defects occur. In spite of this fact there is an interventricular septum

defect present, the cause of which is probably due to the fact that the very narrow truncus septum was not large enough to close the large interventricular opening. According to Mönckeberg,[1] the developmental anomaly of the truncus septum, causing aortic stenosis, is accompanied at the same time by defects of the interauricular and interventricular septa.

The ductus arteriosus remained wide open. In this case the high blood pressure, existing in the pulmonary artery, might have some relation to it. But the fact that a high blood pressure itself would not be sufficient to cause a patent ductus arteriosus will be demonstrated by the second case. We must agree with Mönckeberg that, in this and similar cases including isthmic stenosis, both anomalies occur independently but in coördination one with the other.

The blood circulated in the following way: From the large veins the venous blood emptied into the right auricle and from there into the right ventricle. Through the patent foramen ovale and interventricular defect a part of this venous blood passed into the left auricle and also into the left ventricle. From the pulmonary artery a portion of the venous blood ran into the branches of this vessel; the other portion emptied through the ductus arteriosus into the descending aorta. The oxygenized blood, coming from the pulmonary veins, filled the left auricle and afterwards the left ventricle but it was mixed with venous blood through the defects. This mixed blood supplied the aorta, innominate and carotid arteries. Therefore, the head and right arm received a mixed blood, the other organs a venous blood supply.

In spite of the fact that the blood supply was very poor it did not cause serious trouble and the life of the child was somewhat prolonged.

Summary

A rare anomaly of the heart is described — the entire absence of the aortic arch, which occurred because, in embryonic life, the fourth left branchial artery was not laid down. The rarer type of aortic stenosis was also found with an interventricular septum defect. The congenital heart anomaly had no relation to death, the cause of which was a general malnutrition and bronchopneumonia.

II. Isolated Transposition of Ventricles, Accompanied by the Origin of both Aorta and Pulmonary Artery from the Left Ventricle, and by Stenosis of the Aorta

There are but a few cases in the literature reporting both the aorta and the pulmonary artery arising from the right ventricle. The development of this anomaly may be sufficiently explained by the incomplete developmental course, namely the defective twisting of the septum trunci.

Still rarer are the cases in which the large arteries originate together from the left ventricle. We thought it therefore worth while to describe the following case, as we could not find a similar one in the available literature. The other associated anomalies also added an interesting feature to this case.

Case II

Clinical History: Newborn, white, male infant. In the first forty-eight hours of extrauterine life no abnormalities were found. On the third day the heart-beats and breathing became rapid. The same night the baby became cyanotic and was breathing very rapidly.

On the fourth day X-ray examination showed enlargement of the heart and a possible enlargement of the thymus gland. Otherwise the heart-beats were not so frequent as normal, but a faint murmur was heard. The respirations were very rapid and shallow on the afternoon of the fourth day, 60 to 80 per minute, and the cyanosis was marked. This condition was soon followed by death.

Necropsy Report: (No. A. 27–34.) The body of a well developed, newborn, white, male infant. The skin, especially that of the face and neck, is congested. Lungs, liver, spleen and kidneys are congested. The heart is enlarged, due to the left ventricle which forms the wide and round apex, while the right ventricle seems to be very small. Externally it is noticed that the pulmonary artery is wide and that it is connected with the descending thoracic aorta by the thick ductus arteriosus. The ascending aorta is narrow and increases at the arch up to the point where the ductus arteriosus joins causing a conical isthmic stenosis. The right coronary artery appears very wide (Fig. 5). The measurements of the heart are as follows: length 6 cm., width 4.5 cm., sagittal diameter 3 cm. The left ven-

tricle is dilated and the trabeculae are flattened, the wall is 5 mm. in thickness, but at the apex only 3 mm. The right ventricle seems to be contracted to the limit, the wall being 6 to 7 mm. thick but the lumen only a crevice-like space. The heart muscle is grayish yellow, especially subendocardially in both ventricles. Both auricles are dilated and connected by the 7 by 5 mm. wide-open foramen ovale. The venae cavae superior and inferior empty into the right, and the pulmonary veins into the left auricle. The right venous orifice is narrow, 4 mm. in diameter and has two very small cusps, 2 by 2 mm. broad, one anterior and one posterior. No artery originates from this ventricle. The left venous orifice is wide, 18 mm. in diameter, and its valve has three cusps, one posterior, one medial and one anterior, which are in accordance with the size of the orifice. Both pulmonary artery and aorta arise from this ventricle, the pulmonary artery being situated at the left in front, the aorta somewhat to the right and posterior to it. The opening of the pulmonary artery is wide, 13 mm. in diameter, while that of the aorta is narrow, being only 4 mm. (Fig. 4). The truncus septum ends free in the left ventricle with a downward concave edge, and it is adherent to the medial valve. At the right end of the septum trunci there is a black pepper-sized membranous mass with a pinpoint-sized opening. Through this opening, one can pass a probe into the right ventricle. The dilated trunk of the pulmonary artery gives its main branches to both lungs but the ductus arteriosus, which has a lumen 2.5 mm. in diameter, is entirely obstructed by a thrombus slightly adherent to the wall.

There are right, left and anterior semilunar pulmonary valves, and right, left and posterior aortic valves. The former are large, the latter small. Both coronary arteries arise from their usual position. The left one is very thin and small. The right one has a large opening above the right semilunar valve and at first turns to the right, running for a while in the auriculoventricular groove. Arriving at the posterior interventricular groove it bends toward the apex but does not reach it. It appears very large externally and shows an aneurysm-like dilatation. Several cross-sections were made through the right ventricle, and it was found that the wall of the right coronary artery was very thick. Its lowest part, which shows the aneurysm-like dilatation, is directly connected by a wide passage with the small, crevice-like cavity of the right ventricle.

Histological examination shows that the right coronary artery has a thickened wall with a thickened intima. At the point where this artery is connected with the right ventricle its wall gradually diminishes and disappears. In the ductus arteriosus there is an organizing thrombus occluding about half of the lumen. On top of this there is a recent thrombus which is obstructing the entire lumen of the ductus. In the lungs there is a hyperemia and the capillaries are very much dilated and filled with red corpuscles.

Summarizing the anatomical situation in the heart, we find: (1) both pulmonary artery and aorta arising from left ventricle; (2) aortic stenosis; (3) isthmic stenosis of aorta; (4) thrombosis of the previously dilated ductus arteriosus; (5) tricuspid valve on the left, bicuspid on the right of the interventricular orifice; (6) an unusual course of the large right coronary artery; and (7) an open foramen ovale.

Discussion

In the early stage of embryonic development the truncus arteriosus is situated on the right side of the curved heart tube. Therefore, the origin of the pulmonary artery from the right ventricle is already given at that time. The origin of the aorta from the left ventricle is due to the rotation of the septum trunci, but the entire separation occurs only with the development of the membranous part of the interventricular septum. In the present case there is a tricuspid valve present in the left ventricle, and a mitral valve in the right, while the auricles have a normal position. The position of the truncus arteriosus was therefore on the left side in embryonic life. This was due to the fact that the portion of the heart tube from which the ventricles are formed twisted in just the reverse way to the normal procedure, but the auricles kept their usual arrangement. This is an isolated transposition of the ventricles with a tricuspid valve in the left and with a mitral valve in the right venous orifice. Accordingly, the origin of the truncus arteriosus from the left ventricle took place in embryonic life. The fact that both large arteries originate from the left ventricle is the result of a developmental anomaly of the septum trunci. Therefore we found the more frequently occurring type of aortic stenosis. The septum trunci, which in stenosis divides unevenly the truncus arteriosus, may twist from left to right 225 degrees. This is the rarer form of aortic stenosis

(Fig. 6*a*). Under such circumstances each artery originates from a different ventricle. Such an anomaly was presented with the first case. The other type of aortic stenosis is formed by the rotation of the septum trunci through only 135 degrees in the usual direction instead of 225, causing the pulmonary artery to remain left anteriorly, the aorta right and posteriorly (Fig. 6*b*). This latter anomaly occurred in the present case. The truncus septum is practically in a perpendicular plane to the interventricular septum and the pulmonary artery runs almost parallel with the aorta. The twisting direction of septum trunci is normal and the pulmonary artery should originate from the right ventricle, but due to the unsatisfactory rotation it arises from the left ventricle together with the aorta. This unsatisfactory twisting is most commonly the cause of the wrong origin of the large arteries (Mönckeberg).

The conical isthmic stenosis of the aorta is in close connection with the aortic stenosis. A lesser amount of blood was circulating in the stenosed aorta and this portion adapted itself to the smaller amount of blood. The descending thoracic aorta soon reaches its regular size.

The pinpoint-sized defect in the ventricular septum membranaceum represents the typical subaortic defect and it is only very small. The pars muscularis of the anterior part of the interventricular septum is well formed. The defect is surrounded by a ring-like membrane, which is formed by the septum intermedium. Complete closing could not be performed, because this is done by the septum trunci, which must have the same plane as the interventricular septum.

The wide and thick ductus arteriosus is obstructed by a thrombus. One part of the thrombus shows organization, but the other is very recent, occurring probably only in extrauterine life.

In a case of isthmic stenosis of the aorta the ductus arteriosus is usually opened and wide. It is evident that the increased blood pressure has some relation to the widening and prolonged opening of this passage. But that it is in itself not sufficient to cause it we can demonstrate with this case. The blood pressure in the pulmonary artery was undoubtedly higher than normal because the whole amount of the blood of both auricles emptied in the left ventricle. From here a larger part of the blood passed in the pulmonary artery and, subsequently, in the ductus arteriosus increasing the pressure.

In spite of this the ductus is obliterated. We can explain this only by the fact that when in the development of the ductus there is no anomaly the obliteration by thrombosis will occur even against an increased pressure.

The patent foramen ovale is in close relationship to the increased blood pressure existing in the right auricle, while in the left auricle the pressure is much less.

The right coronary artery is markedly large, and its direct connection with the right ventricle is hardly explainable.

The blood supply of the body in this case was supplied always by means of a mixed blood, but it was fairly satisfactory. When the ductus arteriosus became absolutely obstructed (which was practically the beginning of the dangerous symptoms) the large blood circulation received only a part of the necessary blood. This amount was not sufficient to furnish enough oxygen, and cyanosis developed which was increased by the slow passing venous blood. The left ventricle was supplied with a very small coronary artery, degeneration developed, and with the dilatation of this ventricle death occurred.

Summary

A congenital heart anomaly is presented, in which an isolated transposition of ventricles occurred. Both large arteries, the aorta and the pulmonary artery, arise from the left ventricle. This is explained by the imperfect rotation of the septum trunci, which divided the truncus arteriosus, causing an aortic stenosis. A very small subaortic defect was found. The independent behavior of the ductus arteriosus and its importance in relationship to the dangerous results of the case are also pointed out.

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DESCRIPTION OF PLATES

PLATE 57.

FIG. 1. Absence of one portion of aortic arch, aortic stenosis, and descending thoracic aorta arising from pulmonary artery.

FIG. 2. View of the wide pulmonary artery, right ventricle and interventricular septum defect.



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PLATE 58.

FIG. 3. (*a*) Arrangement of the branchial vessels in embryonic life.

(*b*) Normal development of the persistent arteries.

(*c*) Diagram of the developmental anomaly occurring in the first case.

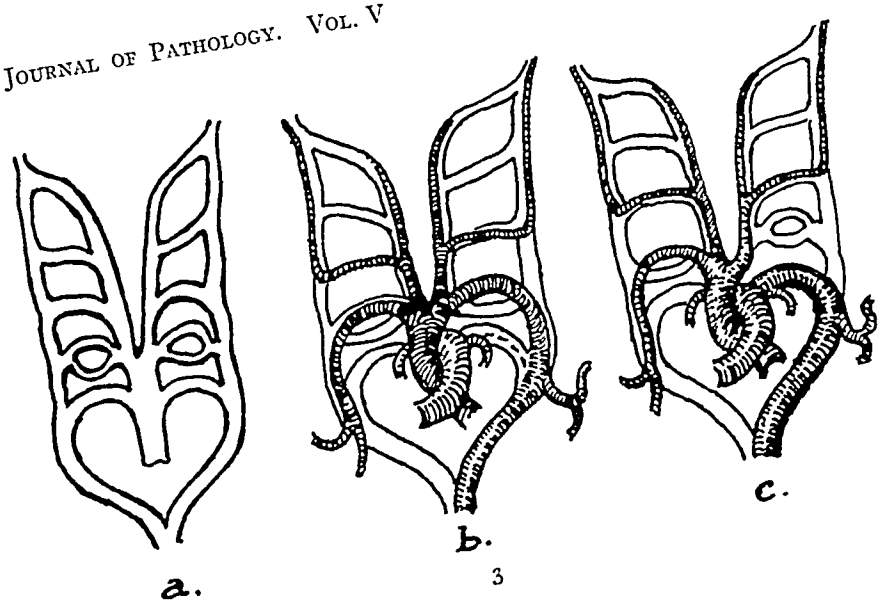
FIG. 4. Aorta and pulmonary artery arising from the left ventricle and stenosis of aorta.

FIG. 5. The wide right coronary artery as seen from the diaphragmatic surface of the heart.

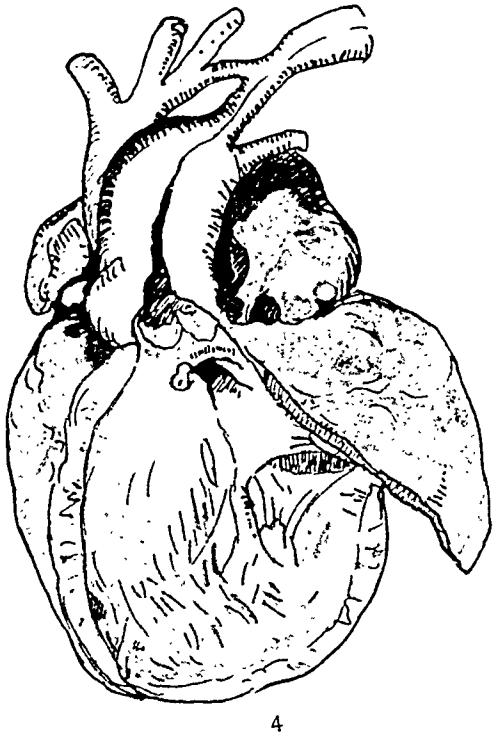
FIG. 6. (*a*) Diagram showing rotation of septum trunci in the rarer form of aortic stenosis.

(*b*) Rotation of septum trunci in the frequent form of aortic stenosis.

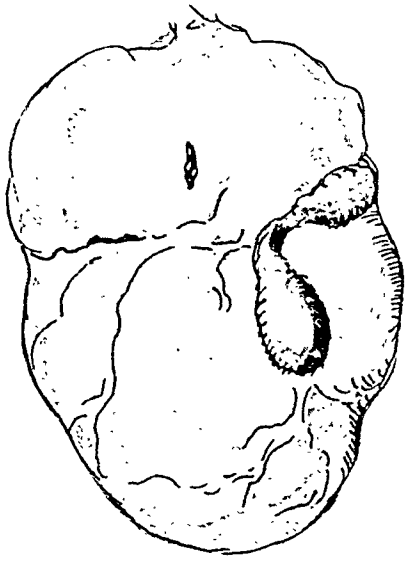
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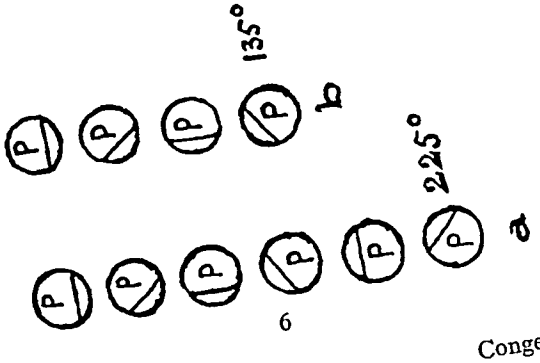
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Congenital Anomalies of Heart

Gáspár

STUDIES ON SEX. INTERSTITIAL CELLS IN GIGANTISM *

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A survey of the endocrine literature reveals the fact that the interstitial cells of the testis (Leydig) are generally credited with a definite internal secretion responsible for limiting growth and for producing secondary sex characteristics of the masculine type. These cells, while probably of connective tissue origin, differ markedly from the connective tissue cells proper to be found in the spaces between the tubules, Whitehead,[1] Kaufman,[2] Boring and Pearl,[3] Mott,[4] Eberth,[5] Freiberg,[6] and others. They are large, oval, epithelioid cells containing numerous crystalloids of variable size and staining qualities, and susceptible of stain differentiation from the surrounding connective tissue cells (Figs. 1, 2, 3, and 4).

It has been supposed (Kaufmann,[2] Bouin and Ancel,[7] Barker,[8] Bramwell,[9] Geddes,[10] Kasai,[11] Lipschütz,[12] Griffiths,[13] and others) that gigantism with feminism was probably due to absence or change of the secretion of the interstitial cells. For this reason, it is felt that a report of the following case may be of some interest.

While making a study of the dental conditions in the endocrine cases among the patients of the Michigan Home and Training School at Lapeer, Michigan, in the fall of 1927, I was permitted to examine and to obtain a relatively complete history of a male patient in whom gigantism was not coupled with definite eunuchoid or feminoid characteristics. Upon the death of this individual, it was my privilege to make a partial postmortem examination, including a number of X-rays, and to obtain one of his testes for microscopic study.

The subject, a feeble-minded patient, died from an acute intestinal obstruction, apparently not connected with his supposed endocrine condition. At death, he was 47 years old. His genetic history, as far as could be ascertained, was negative. The only feature worthy of note was the fact that he had meningitis during his tenth year. Prior to that time, it had been noted that he was not of average mentality, but nothing of a somatically abnormal nature had been re-

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marked. His increase in stature, together with the hyperplastic condition of his genitalia, date apparently, from that infection. His height was 79½ inches. He had grown 5½ inches since the age of twenty-one, at which time he had been admitted to the institution. His head circumference was 24¾ inches at the level of the frontal prominences, which were quite marked. His chest measurement was 38½ inches. The penis from symphysis pubis to end of glans was nearly 8 inches in length in the postmortem condition, and the testes and scrotum were proportionately large. From the crest of the ileum to the soles of his feet measured 44 inches. His hip measure was 47 inches. It will be seen from these figures that he was slightly macrocephalic and only a trifle, if at all, feminine in contour. These minor variations were in turn offset by the fact that his external genitalia were decidedly hyperplastic and that, inasmuch as he was a constant and frequent masturbator, there certainly was no marked decrease in his libido. Neither was there any definite mark of feminism about his facies or hairy development, unless the fact that his beard was limited to the chin and the lower anterior portion of his face might be so considered. Except for their size, the X-ray disclosed his long bones to be of about normal contour and density, merely showing a definite epiphyseal line. The pituitary, while it could not be examined microscopically, did not appear abnormal on X-ray.

Microscopic examination of the testis revealed the tubules and their contained cells well within the bounds of the average for the patient's age, consequently safely classed as normal. There was a notable increase in the amount of interstitial connective tissue. While there were cells present in the interstitial spaces, they were certainly not normal "interstitial" cells. These cells were throughout spindle-shaped, lacking the clear ovoid outline, both of nuclei and cells proper, shown by typical interstitial cells. They lacked also the granular cytoplasm and the nuclear chromatin net shown by the interstitial cells of Leydig. In many cases they were larger than the connective tissue cells usually are, but, due to their form and architecture, I believe them to be only connective tissue elements.

The case is very largely that of fibrosis testis as described by Kuntz [14] and others, except that these authors do not mention a complete lack of interstitial cells (Figs. 1, 2). Oslund [15] thought there was an interchangeability of the two types of cells under vary-

ing conditions. It is not impossible that in the case which is the subject of the present study, the normal interstitial cells may have been present up to and following puberty, possibly until the age of 21 years, when the later skeletal overgrowth became apparent. Toxicity or exhaustion due to frequent masturbation could conceivably be the cause of their disappearance and the later appearance of many symptoms generally attributed to dysfunction of these cells.

One would expect to find as the result of an absence of interstitial cells, in addition to possible gigantism, definitely feminine secondary sex characteristics and a marked diminution of the libido. Were dyspituitarism the cause of the condition, as has been indicated by Cushing [16] in cases in many ways similar, one would still expect to find the feminine type of sex characteristics and the decreased libido (Hutchinson,[17] Frank,[18] and others). In addition to the fact that neither of these conditions existed in this case, one must note the presence of hyperplastic genitalia and no evidence of delayed closure of the epiphyses, all of which would seem to eliminate the pituitary.

There is no scarcity of literature to demonstrate the effects of castration upon the development of the bony skeleton or upon changes in the secondary sexual characteristics (Myers,[19] Moore,[20] Kuntz,[14] Dixon [21]). The removal of the testes entirely, undoubtedly appears to remove from the body some hormone which affects these conditions to a marked degree. However, the opinion that this hormone is the product of the interstitial cells solely does not seem to be entirely warranted. In addition to the fact that such a secretion might come from other testicular cells, it must be remembered that castration involves the loss of what is probably the most important function of the organism, that of procreation. It is scarcely to be believed that such an interference could take place without an observable reaction in the organism, even should this reaction be largely or wholly psychic. Doubtlessly, a hormone or hormones are involved, but it is hardly necessary to limit the effect of castration to their action.

Myers,[19] in studies upon vasectomized rats, compared the effects of vasectomy with those of castration. He noted that in vasectomy no marked physical or psychical changes resulted, and also that there was no observable change in the interstitial cells. On the other hand, spermatids and spermatozoa degenerated. Following castration there were marked changes of a somatic and psychic nature,

i. e., reversion to feminoid physical type and unstable mental condition. His very logical conclusion was that the interstitial cells controlled these conditions. He did not remark upon the Sertoli cells or the spermatogonia, but it is true that vasectomy does not markedly alter these cells, and it is at least possible that either or both may secrete the hormone or hormones involved. The same considerations apply to the work of Hanes [22] as to that of Myers just mentioned. However, Boring and Pearl [3] in a rather exhaustive study of the interstitial cells under varying conditions, felt that their appearance was so irregular that no definite endocrine function could be safely ascribed to them. Oslund [15] found that vasectomy had little or no effect upon any of the testicular cells, and that experimental cryptorchidism produced atrophy of sex cells, but hypertrophy of interstitial tissue. While Des Cilleuls,[23] like Myers, Hanes, and others, felt that the case for the endocrine character of the interstitial cells was made, the later more detailed work of Boring and Pearl, using the same type of animals as Des Cilleuls, seems to weaken the nature of his conclusions.

Warthin [24] from many years of careful clinical and laboratory observation, feels that the weight of the evidence is in favor of the function of the cells of Leydig being mainly a nutritive one. Bramwell,[9] Cushing,[16] P. E. Smith,[25] Van Wagenen [26] and others, approaching the study from both laboratory and clinical angles, have felt that conditions of the sort mentioned, could be ascribed to the anterior lobe of the hypophysis, especially as regards the variations in bony growth. My own work on dogs [27] would tend to confirm this.

Frank considered that due to the immensely conflicting nature of the evidence no positive conclusions could be drawn as to the effect of either interstitial cells or pituitary, from either the published experimental or clinical observations. Kasai in a particularly complete study on a long series of human autopsies found such wide variations in both interstitial and intratubular cells in individuals coming well within normal limits, as well as those of a distinctly abnormal make-up, that he was unwilling to draw conclusions as to the possible endocrine function of either of these cell types. Dürck [28] found no regularity in the appearance of the interstitial cells in varying conditions, unless it was a tendency toward increasing paucity as the result of debilitating disease. This same tendency was remarked by Mills [29] in his studies of the testes of patients dying in the army

camps from pneumonia and influenza. The former of these writers (Dürck), also cited one of the few cases to be found in the literature of complete absence of interstitial cells, but his case, totally at the opposite extreme from that reported here, was a cretin, with hypoplastic genitalia. Dürck's case and mine, diametrically opposed in build and the sex characteristics, but both presenting an absence of interstitial cells, would argue against the rôle of these cells in influencing either or both of these conditions.

Hosftätter [30] was unable to influence the testicular cells to a definite degree by the transplantation, injection, or feeding of pituitary preparations.

Lipschütz,[31] in his exhaustive study of the sex glands, found that the adult interstitial cells came into prominence at about the period of puberty. He also noted that continued intoxication of any kind caused their atrophy. Finally, he concluded that the evidence was in favor of these cells being endocrine in their nature, although he admits that he does not consider the case definitely or finally proved. His findings regarding both their time of appearance and conditions causing their atrophy would be fully as apt to apply to cells of a purely nutritive or supporting character as they would to endocrine cells.

Sand [32] and Kuntz found that frequently there was an increase in the intensity of sexual ardor coincident with hyperplasia of the interstitial cells. On the other hand, the case described here argues that the absence of these cells by no means necessitates a diminution in that intensity.

Tsuji [33] demonstrated that thyroid extracts had a definite effect upon all of the testicular cells, but apparently did not feel that that proved their endocrine character. Freiberg accepted the belief that the interstitial cells were definitely endocrine and with that assumption made a study of their changed appearance under varying conditions.

Hudovernig and Popovits [34] and Hudovernig [35] reported a case carefully studied clinically over a period of years. Their case is so markedly similar to the case which is the subject of this study, that they may be considered as similar in type and possibly in etiology. By the method of trial and error, they were finally able to improve this patient's condition by the administration of *ovarian* extracts. This in spite of the fact that the patient was a male. This would

seem to argue against the specificity of any hormone secreted by the testicular interstitial cells.

On the whole, the more complete the review of the literature on this subject, the more apparent it becomes that there is a complete lack of accord among the investigators who have studied the interstitial cells. The general trend of belief is that they constitute an endocrine organ, although very few workers will take this stand with any degree of positiveness. On the other hand, there is slight evidence to point toward their nutritive character, although again, no conclusive studies resulting in this view have thus far been submitted.

In eunuchoidism, it is remarkable that, although the secondary sex characteristics are apt to be affected markedly, and that frequently there is an increase in stature, there is a possibility of normal interstitial cells, or even of hyperplasia of these cells. In vasectomy, psychic and somatic qualities remain apparently normal and there is little, if any, change in the interstitial cells. In individuals with normal physical and sexual characteristics all variations of the interstitial cells have been noted. The giant may show complete absence of interstitial cells and so may the cretin. Coincident with hypoplastic genitalia, there may be an increase in these cells, while the same condition may prevail in the individual with hyperplastic genitalia. Considering this complete lack of any sort of regularity in their appearance in the various types of physical and psychical aberrations or even in normal cases, it is extremely difficult to attribute to these cells any very important endocrine character.

Summary

1. The case presented here, a definite giant, did not have the hypoplastic genitalia or the marked feminine secondary sex characteristics usually associated with such a condition.

2. Microscopically there was an apparently complete absence of testicular interstitial cells, which, while they are supposed to limit bone growth, are likewise supposed to produce, in part at least, the masculine type of secondary sex characteristics.

Note: I wish to express my indebtedness to Drs. W. J. Kay and M. O. Blakeslee, of the Lapeer Institution, and to various members of the staffs of the Universities of Michigan and Pennsylvania, in particular, Dr. Geo. S. de Renyi, who gave me such kind assistance in preparing the illustrations.

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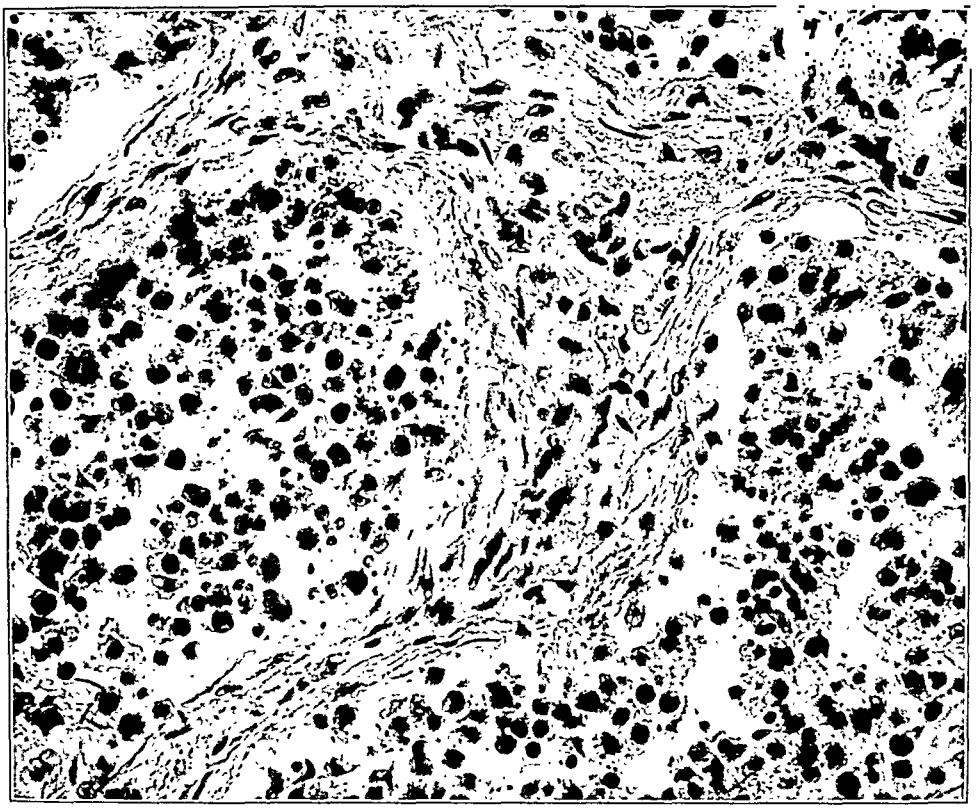
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DESCRIPTION OF PLATES

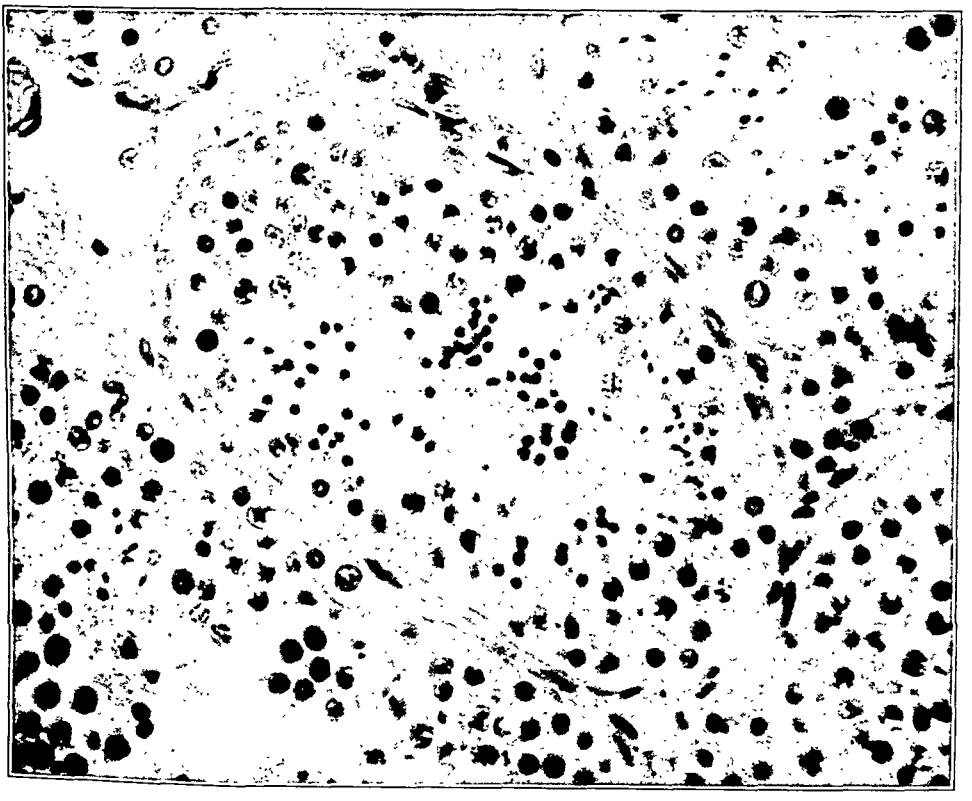
Plate 59.

Fig. 1. Photomicrograph of testis of case cited, showing great increase in quantity of stroma, but no normal interstitial cells (Leydig). × 360.

Fig. 2. Photomicrograph of normal human testis, showing numerous interstitial cells. × 360.



1

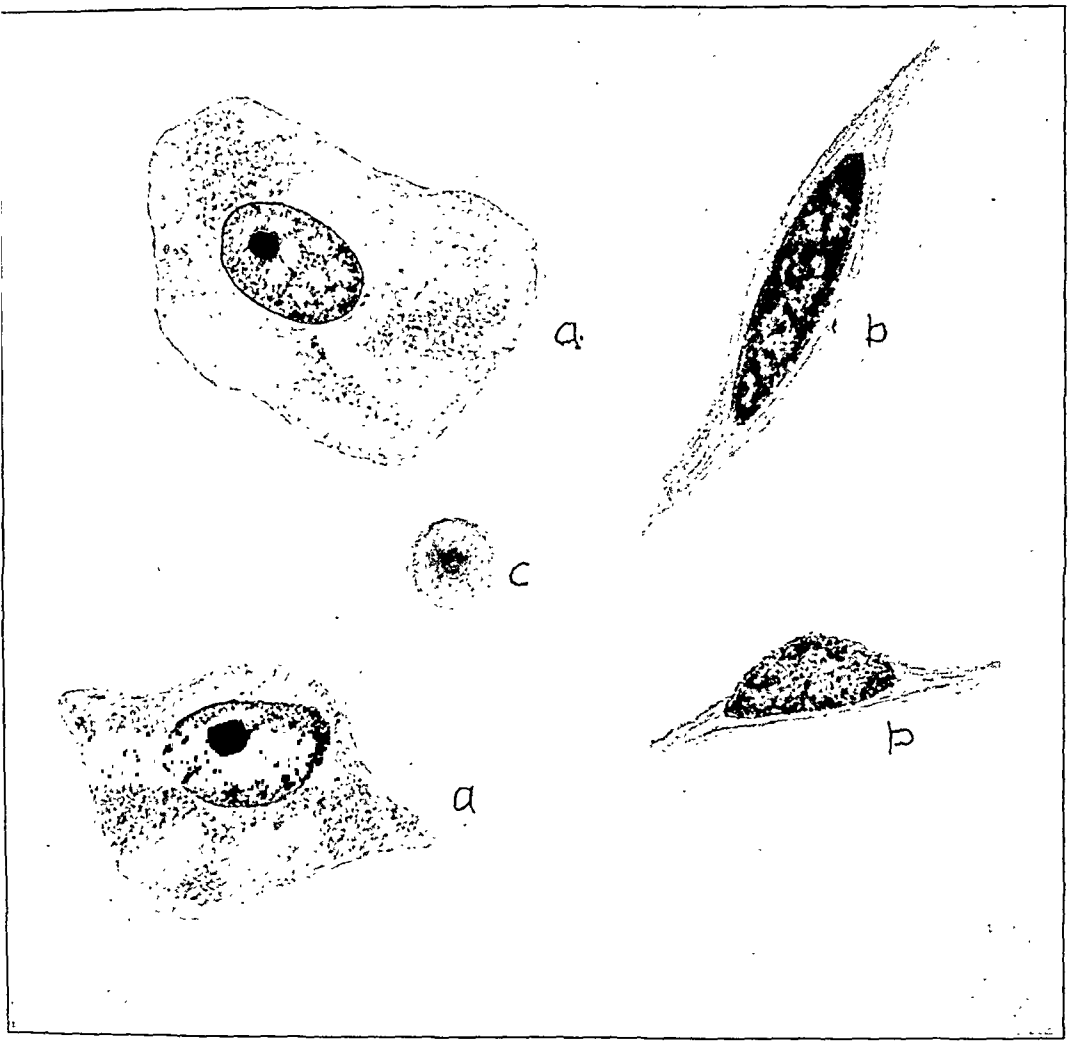


2

PLATE 60.

FIG. 3. Camera lucida drawing of interstitial cells from a normal testis (*a*); connective tissue cells from stroma of case cited (*b*); an erythroycte (*c*), for comparison of size. × 1600.

FIG. 4. Retouched photomicrograph of normal testis showing interstitial cells (*a*); connective tissue cells (*b*); (*c*) a normal polymorphonuclear neutrophile. × 1500.



3



4

MYELOBLASTIC SARCOMA OF THE CRANIUM *

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The proper classification of newgrowths composed essentially of myeloid tissue and its supporting stroma has been the theme of many discussions and lengthy reports. Whether the multiple myelomas, for example, are true tumors or simply narrow tissue hyperplasias and, in that sense, systemic disorders, is a question about which there is no unanimity of opinion. Theories in regard to Sternberg's leukosarcomas, the chloromas, and other related diseases of the bone marrow are equally conflicting, and no interpretation of the nature of these diseases is accepted unequivocally. The purpose of my report, therefore, is not to attempt to arrange these views in some acceptable order by discussing them at length, but rather to place on record an account of a huge solitary myeloid growth of the head with extensive destruction of the regional bone tissues and with metastases into the lymph nodes of both sides of the neck and into the right lung. In all of these places there were cellular structures and tissues which resembled certain components of bone marrow tissue.

REPORT OF CASE

Clinical History: C. B., a negro, aged 52 years, entered the Ear, Nose, and Throat service of Dr. W. H. Theobald at St. Luke's Hospital, September 29, 1928, complaining of pain and soreness over the right eye for about a year, and a swelling for two months. At that time the physical examination disclosed a fluctuant mass about the size of a walnut in the right frontal region, and a purulent exudate escaping from the upper ethmoidal regions on both sides. The pupils were equal and regular but did not react to light. The lymph nodes of the neck were somewhat enlarged and the diagnosis "empyema of the frontal sinus" was made. Dr. Theobald, on October 4th, attempted to drain the frontal sinuses but found only a little exudate in the mass bulging over the eye. He was surprised to discover that the frontal bone was eroded and penetrated, and that the right sinus was filled with gray-white, friable tissue. This tissue extended through the posterior wall to the dura and to some extent filled the left frontal sinus. Tissues removed at this time were considered to be like lymphosarcoma, although the cells in the stroma were much larger than those usually noted in these growths. There were no serious consequences from the operation. The spinal fluid was unchanged. On October 12th, the blood contained 3,680,000 erythrocytes and 9,700 leucocytes per cubic millimeter, and the

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hemoglobin was 69 per cent. The distribution of the leucocytes was as follows: 70 per cent neutrophil and 5 per cent eosinophil polymorphonuclear leucocytes, 20 per cent small and 5 per cent large lymphocytes. On November 4th, the patient complained of dizziness, tinnitus, and diminished vision. A cervical lymph node as large as a walnut was noted under the center of the left sternocleidomastoid muscle on November 6th. A roentgen examination by Dr. E. L. Jenkinson on November 10th, failed to demonstrate metastases of the lungs. By November 13th, both sides of the nose had become occluded by the rapidly growing tissues, and the growth over the right eye had increased markedly in size. On November 21st, the blood contained 2,950,000 erythrocytes and 62,000 leucocytes per cubic millimeter, and 49 per cent hemoglobin. A few leucocytes but no other abnormalities were found in the urine. The tumor mass over the right frontal region grew rapidly and ulcerated in several places. Blindness of the right eye and diminished vision of the left followed. After gradually becoming weaker, the patient died December 9, 1928, at 3.00 A.M.

The postmortem examination of the head, neck, and trunk was started seven hours later.

The essential portions of the anatomic diagnoses are: huge primary myeloblastic sarcoma of the cranium with extensive destruction of the ethmoid, nasal, superior maxillary, frontal and sphenoid bones; marked sarcoma invasion of both orbits; sarcoma metastases of the left submaxillary, the right and left deep cervical lymph nodes, and the right lung; displacement, compression, and superficial invasion of the anterior portion of both frontal lobes of the brain; acute purulent ethmoid, sphenoid, and left maxillary sinusitis; marked emaciation; cloudy swelling of the liver and kidneys; fatty changes of the liver; diminished lipoid content of the suprarenal glands; chronic emphysema, and bronchiectasis of the lungs.

Autopsy Report

Only the pertinent portions of the postmortem record are mentioned. The body of this negro weighed 100 pounds. The left eye was prominent. The nose was flattened and deviated to the left because of a large fungoid mass of tissue that covered the right frontal bone and extended to the left of the midline 4 cm. and upward 10 cm. from the nasion. This mass was 14 cm. in diameter and as thick as 8 cm. It hung down on the right side so as to cover the right eye and maxilla like a huge cauliflower. Three ulcers, each about 7 cm. in diameter, included approximately half of the outside. The tissue in this mass was gray-white, soft and friable like brain substance.

The scalp tissues were reflected in the usual way. Most of the tissue growth was outside of the skull, but in front, corresponding to the right superciliary arch, the bone tables were destroyed.

Brain: The calvarium weighed 450 gm. There was a marked hyperplasia of the myeloid diploe. The bone in front was 7 mm. thick. The dura was closely adherent. The lumen of the superior longitudinal sinus contained fluid and clotted blood; the lining was smooth. The leptomeninges of the brain were thin and glistening. The tip of the left frontal lobe was adherent for a length of 2 cm. to the inside of the dura and to a mass of soft, gray tissue on the left side of the midline. This mass extended upward on the falx 3.5 cm. from the base of the cranium and 3 cm. backward. There was a similar mass of tissue on the right side of the falx, practically as large, and both cribiform plates of the ethmoid were covered over with these tissues, the right slighly more so than the left. The contiguous brain substance was pushed away rather than invaded. The abundant spinal fluid was clear and colorless. The convolutions of the brain were flattened slightly, especially in front, and the sulci narrowed. The olfactory bulbs were destroyed. The stalks were markedly compressed against the brain substance. The anterior poles of both cerebral hemispheres were compressed over areas measuring 4 by 3.5 cm. and to a depth of 2 cm. In removing the brain these tissues were torn superficially and seemed to be invaded slightly with tumor tissue. The leptomeninges between the olfactory bulbs and lateral to these for a width of 2 cm. were slightly opaque, a thickening which continued back to the optic chiasma, covered over the infundibulum, and ended at the anterior margin of the pons. The brain weighed 1,220 gm. The lining of the right and left lateral and sigmoid sinuses of the dura was smooth. When the dura was stripped from the base of the skull a large quantity of thick yellow exudate escaped on both sides from the eroded sphenoid sinus. More was released when the clivus was cut. In removing the dura from the anterior cranial fossa a large amount of friable, gray-white tissue was torn from along both sides of the midline. This tissue adherent to the dura was a mass 7.5 cm. long, 6 cm. wide, and as thick as 3.5 cm.

Cranial Bones: There was an extensive destruction of the cranial bones, extending forward from the posterior edge of the anterior cranial fossa to the ascending portion of the frontal bone, on the

right side 1 cm. wide, and 2.5 cm. on the left. The anterior 2 cm. of the left orbital plate to the lateral edge were destroyed and replaced by soft, gray tissues, which had grown extensively into the left orbit, although small masses of muscle and fat remained. The left maxillary antrum was invaded, but the antrum itself was filled chiefly with a thick yellow exudate. The ethmoid bones were destroyed, and the right orbit and maxillary sinus contained much tumor tissue. A defect of the frontal bone involving both sinuses and including both tables equally on the right and the left of the midline was 5.5 cm. wide and extended 3.5 cm. into the nasal septum. There were no changes of the middle ears or ear ossicles.

Lymph Nodes and Glands: The right and left axillary lymph nodes were scarcely 1 cm. in diameter. The right and left superficial cervical lymph nodes were small, flat, and dark red. The right submaxillary lymph node was gray-pink, 1.5 cm. in diameter; and the left, shaped like a pigeon's egg, measured 4 by 2.7 by 2.5 cm. and consisted largely of white, opaque, friable tissue. Both submaxillary salivary glands contained coarsely lobulated brown tissue. In the left posterior cervical triangle near the apex, lying beneath the sternocleidomastoid muscle, was a large lymph node shaped like a goose egg, 8 by 5 by 4.5 cm. It extended to the base of the cranium. Around this were several other small lymph nodes, and the medial edge of this mass of lymph nodes was in direct contact with the internal jugular vein. The lining of this vein was smooth. These masses contained soft, white, opaque tissue, mottled 50 per cent with small irregular regions of necrosis. At the apex of the right posterior triangle were other lymph nodes, as large as 2.5 cm. in diameter. They were along the internal jugular vein, but the lining of this vein was smooth. These nodes extended to the base of the cranium. The muscles of the neck were unchanged. The thyroid gland, weight 19 gm., was symmetrical. There were no enlarged lymph nodes along the front of the cervical spine. The oral surface of the hard and soft palate was smooth mucosa and the tonsils were small. The lymph nodules at the base of the tongue were pink. There were no noteworthy changes of the pharynx, esophagus, epiglottis, trachea or larynx. The tracheobronchial lymph nodes were 2 by 0.7 by 0.5 cm. and contained chiefly soft black tissue.

Lungs: In the upper lobe of the right lung 6 cm. from the apex was a mass of gray-white tissue 1 cm. in diameter. There were no other similar masses.

Other Tissues: The routine examination of the trunk included the innominate veins, superior and inferior venae cavae and their main branches, heart, aorta and its main branches, thoracic duct, parathyroid glands, liver and its bile ducts, kidneys and ureters, pancreas and the duct, spleen, stomach and bowel, prostate, seminal vesicles, urinary bladder, vasa deferentia, testes, epididymides, biliary and mesenteric lymph nodes, rectum, spine, ribs, and the bones in a general way.

Pathological Examination

Because of the changes due to necrosis in the primary growth and in order to avoid confusion with tissues into which the tumor had grown, the lymph node metastases were considered best suited for study, and the tissues in these places were then compared with the tissues in the primary growth. These tissues were fixed in Zenker's solution and in formalin. Thin sections prepared by the paraffin method were stained with hematoxylin and eosin, phosphotungstic acid hematoxylin, May–Grünwald according to Helly,[1] Giemsa (Wolbach's modifications), and iron hematoxylin. Alcohol-fixed material was stained for glycogen.

Lymph Node Metastasis: The lymphoid tissue of the left deep cervical lymph node was entirely replaced by tumor. The appearance of the tumor varied considerably because of the amount of stroma and necrosis. In arranging the description, therefore, it seemed logical to describe first those places with practically no stroma and then those with increasing amounts of connective tissue.

Sections with Little Stroma: The highly cellular masses of tissue were arranged around thin-walled blood channels in masses as large as 1 mm. in diameter. These consisted of large cells, 20 to 25 microns in diameter, closely grouped without stroma like a mosaic, the boundaries, fine linear tracings corresponding to membranes of contiguous cells (Fig. 1). The outline of all of these cells was not the same, but the variations were such as occur when flexible globules are crowded together. The abundant cytoplasm was granular and vacuolated. The large vesicular nuclei were oval, round, or indented. The

chromatin material of the nuclei was distributed in a few fine threads, in a number of small masses along the nuclear membrane, or in one large or several small round compact masses. The continuity of the mosaic pattern was interrupted by small clefts, usually having two or three times the diameter of the cells mentioned. Some of these were artefacts, while others were probably the result of necrosis or cell shrinkage. In such places there were several polymorphonuclear leucocytes and cellular débris. In addition to these were much larger regions where the cells were much more loosely arranged. With these cells were polymorphonuclear leucocytes, granular débris of cells, and a number of cells with cytoplasm that stained like erythrocytes, some having a few basophilic granules and with a solid or coarsely granular nucleus. Similar cells when found in stained films of blood are considered, without much hesitation, to be normoblasts. The granules in the cytoplasm of the cells in tissues stained according to May–Grünwald and Giemsa (formalin, Zenker, and Schaudinn-fixed material) were generally neutrophilic, but in the mosaic in direct continuity with these cells, without regular distribution, were some with coarse eosinophile granules and a thick horse-shoe-shaped or lobed nucleus. Many of these equalled in size the cells with the neutrophile granules. A few cells had a number of coarse basophilic granules. Along the periphery of these masses the cells were necrotic.

Sections with Much Stroma: Where collagenous connective tissue was more abundant, narrow septums formed small compartments with large, round or polygonal cells of the character mentioned, singly, or in groups of several. The dimensions of the cells in these structural units were somewhat greater than 25 microns. Within some of these alveolar units there was very little supporting stroma, but in others there was more, these transitions passing gradually into tissues with considerable stroma (Fig. 2), in which the cells were separated from each other by narrow fibrils or bands of connective tissue. Special mention should be made of large cells, as much as 50 to 60 microns in maximum diameter, with neutrophilic, granular, and vacuolated cytoplasm, and with one or several large, vesicular nuclei or a single, coarsely lobed, crescentic nucleus as in a megakaryocyte. Among the cells were many in mitosis.

Primary Tumor: In portions of the primary tumor examined (Fig. 3), the stroma was abundant and there were only small masses

of cells arranged in mosaics. Here, too, were many necrotic places, but especially numerous were the large cells of the megakaryocyte (Fig. 4) variety.

Lung Metastasis: The small mass in the lung was histologically the same as the tumor tissue of the lymph node.

Oxidase Granules: Frozen sections of formalin-fixed material (lymph node metastases) were stained for oxidase granules by the Graham's benzidine method [2] for tissues. In the tumor tissue there were many cells filled with mahogany-brown granules. The large myeloblastic cells contained none, or only a few.

Other Examinations: Histological examination of lymph nodes (2) along the greater curvature of the stomach, parabronchial lymph nodes (2), mesenteric lymph nodes (2), biliary lymph nodes (3), left axillary lymph nodes (2), right submaxillary lymph node, tracheal lymph nodes, right and left superficial cervical lymph nodes, periesophageal lymph nodes, and of the tonsils, seminal vesicles, suprarenal glands, myocardium, diaphragm, aorta, gall-bladder, esophagus, parotid gland (3), epididymides, stomach, lungs, liver, kidney, spleen, submaxillary salivary glands, pancreas, testes, thyroid gland, root of the tongue, prostate, urinary bladder, small and large bowel, failed to disclose tumor tissue, or other unusual changes.

Discussion

There is little doubt that the growth in the head is myeloblastic tissue, a conclusion that is logical because of the presence of myeloblasts, eosinophilic myelocytes, megakaryocytes, and erythroblasts. It has the properties of a malignant tumor as shown by the local extensive destructive growth and the occurrence of secondary growths in the cervical lymph nodes and in the right lung.

Borst [3] described malignant tumors which contain immature precursors of myelocytes, leucocytes, and megakaryocytes as myeloblastic sarcomas (myeloma sarcomatodes). Usually there is only one cell form represented; rarely, the particular malignant tumor simulates the entire bone marrow with its different forms in an atypical, incomplete way. Froboese [4] studied an infiltrative and destructive tumor of the sternum that contained erythroblasts, erythrocytes, myeloblasts, and myelocytes, with all their antecedent and intermediate forms. He regarded the growth as one of multiple

myelomas and stated that although certain myelomas are accompanied by visceral metastases most of them are not, and that no one because of its expansive growth is to be interpreted as malignant in the usual sense. Wallgren's[5] extensive review of myelomas cites many accounts of visceral growths with multiple myelomas, although Wallgren himself agrees with the general conclusions stated by Froboese. Ribbert[6] in 1904 described a sarcoma of the head with metastases to the spine, which by postmortem examination proved to be a myeloma. The right orbit was invaded extensively, and other nodules were discovered in the ribs and spine. He found cells in the tumors, like erythrocytes, and other cells transitional between erythrocytes and colorless tumor cells. The report by Roman[7] described two patients with multiple malignant tumors of the bones, composed of myeloid tissues and accompanied by widespread metastases of the lymph nodes. He mentions four other reports which he regarded as similar. Another account by Herscher and Thevenard[8] described two large tumor masses of the skull and another of the sternum. The cells of these resembled myeloblasts, myelocyte precursors, and megakaryocytes.

Summary

A large myeloblastic sarcoma of the skull with metastases into the right and left deep cervical lymph nodes and into the right lung is described in this report.

The tumor and its metastases contained cells that resembled cellular components of the bone marrow.

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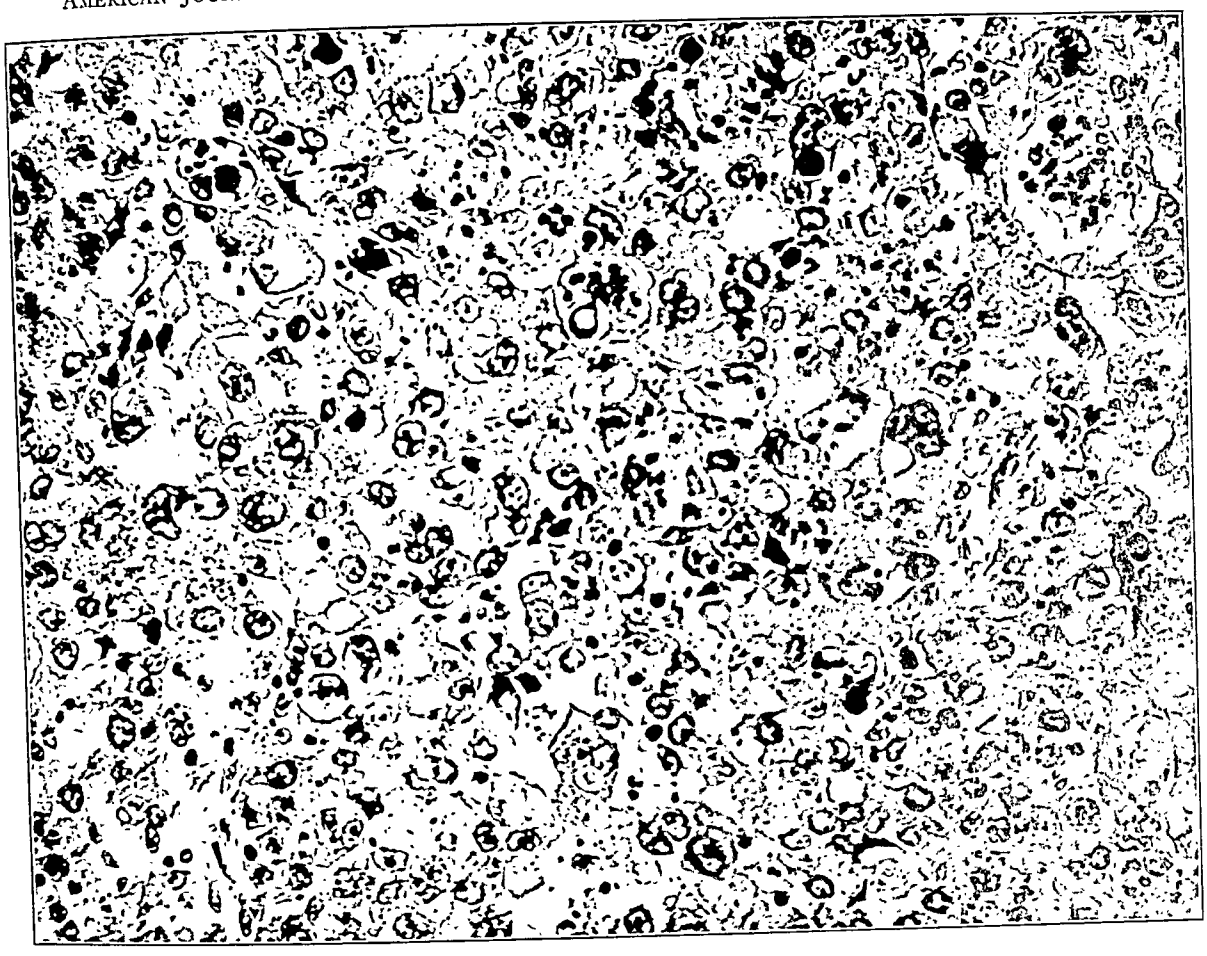
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DESCRIPTION OF PLATES

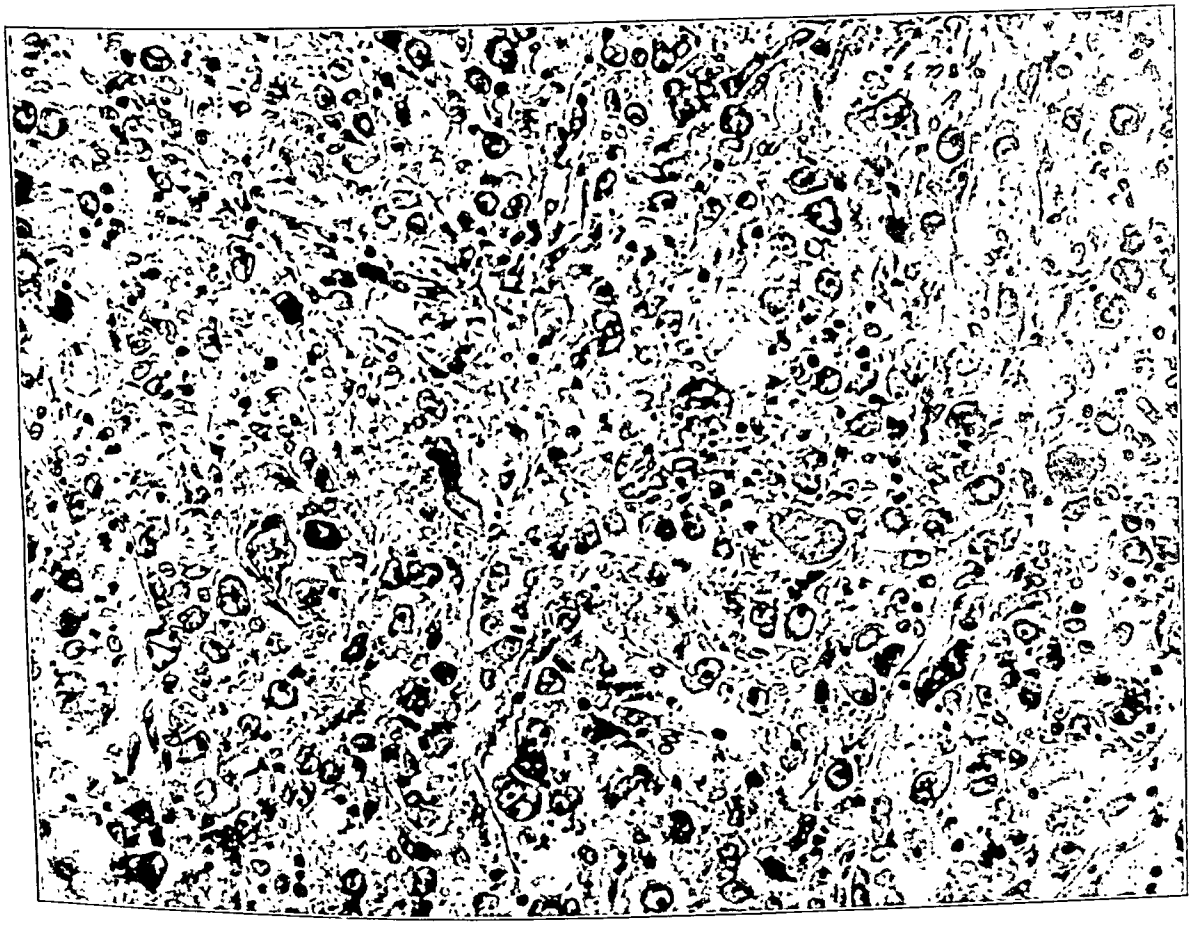
PLATE 61

FIG. 1. Photomicrograph of a lymph node metastasis to illustrate the mosaic arrangement of the tumor cells. × 254.

FIG. 2. Photomicrograph of a lymph node metastasis in portions with considerable fibrous stroma. × 254.



1



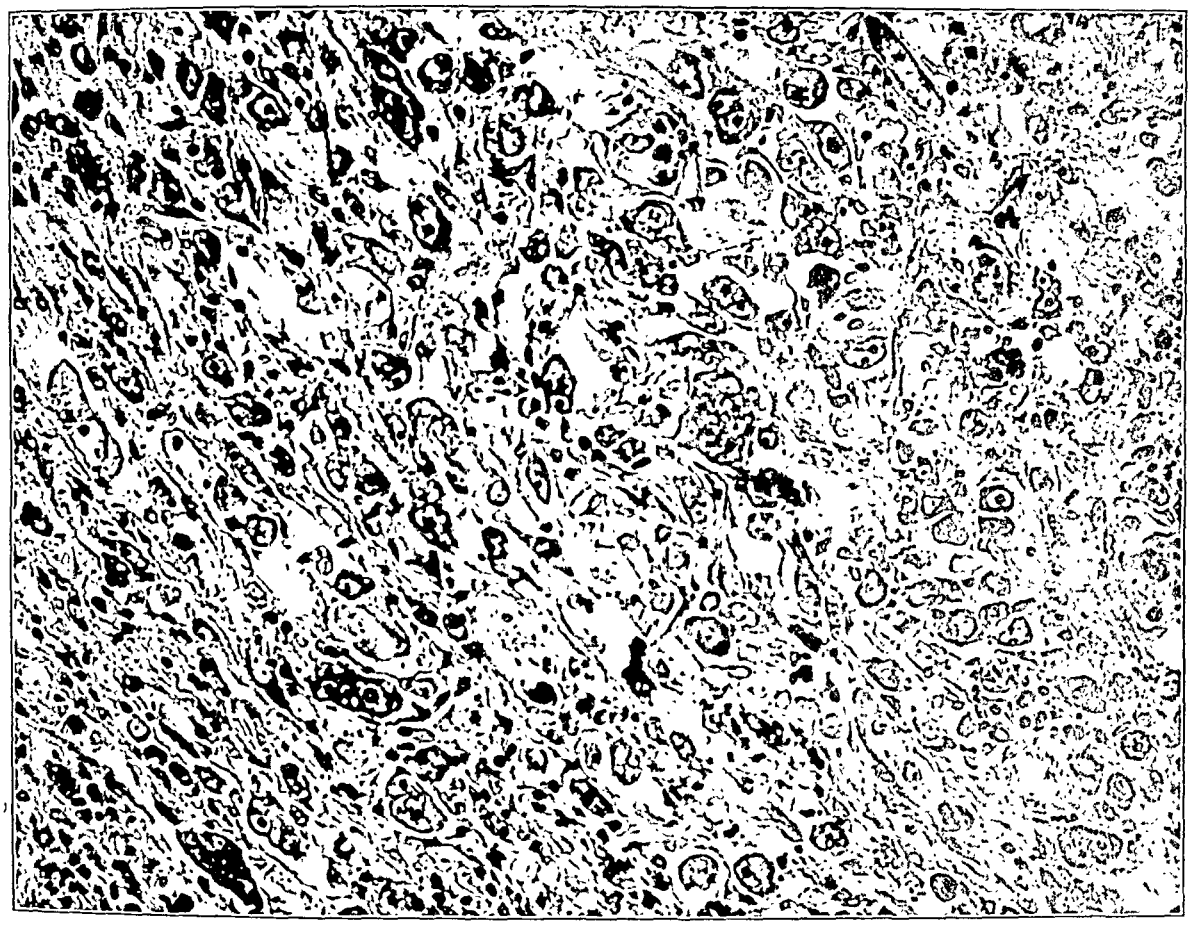
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Hirsch Myeloblastic Sarcoma of Cranium

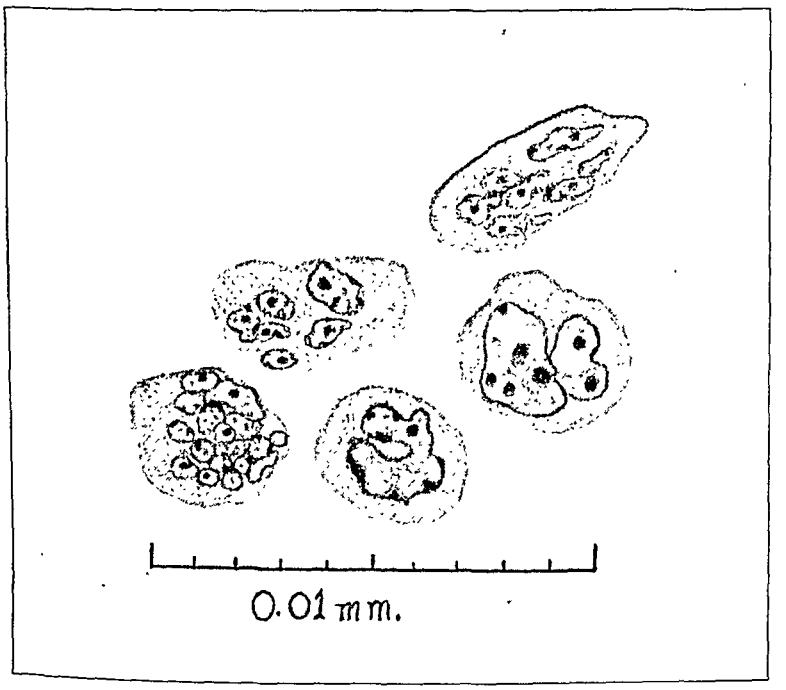
Plate 62

Fig. 3. Photomicrograph of the cranial tumor tissues in which there were many cells of the megakaryocyte variety. × 254.

Fig. 4. Camera lucida drawing of several of the large multinucleated cells in the primary tumor.



3



4

TERATOMA OF THE NECK *

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Teratoma of the neck is rare and deserves a detailed study. A case of this type, on which an autopsy was performed at the City Hospital of Cleveland, served as the basis of this study.

In a review of the literature on this subject it is somewhat difficult to decide whether certain reported cases of so-called teratoma of the neck are true teratomas or not. This is especially true of cases which were reported at a time when the use of the microscope was not yet a routine procedure. There are cases classified as teratoma which either were not satisfactorily studied or showed only derivatives of one or two germinal layers and therefore do not deserve to be so classified. We, therefore, thought it wise to differentiate strictly between teratoma and tumors which possibly might be teratoma, but might be only mixed tumors, and should not go into the literature as true teratomas. Most of these tumors are in close relation to the thyroid. The absence or presence of the thyroid gland determines whether such a tumor shall be called teratoma of the neck or teratoma of the thyroid. If the thyroid gland is absent, the tumor is called a teratoma of the thyroid. If the thyroid gland is present, it is called a teratoma of the neck.

The first case of teratoma of the neck described was that of Grass in 1691. This was a tumor of the neck of a woman and was classified as a fetus. Joube, in 1759, described a tumor of the neck which contained cartilage and bone. This tumor was supplied by branches of the left carotid artery. Morand, in 1776, found hair and intestines in a tumor of the neck. A more detailed description is found in the article of Bury, who, in 1834, published the case of a still-born child with a tumor of the scalp, face and neck. The tumor was called an imperfect fetus containing amniotic fluid, skin, hair and bones.

The earliest four cases, usually accepted as teratoma of the neck, in our opinion do not deserve to be classified as such, even though there is not much doubt about their true nature. The lack of more detailed study prevents us from classifying them as teratoma.

* Received for publication March 21, 1929.

Table I gives the cases of teratoma of the neck; it gives the name of the author, the year of publication, the age and sex of the patient. It further states whether or not the thyroid gland is present in these

TABLE I

Author	Year of publication	Age	Sex	Thyroid gland	Brain tissue	Remarks
Hess	1854	NB	M	pr.	n.r.	
Zahn	1886	SB	n.r.	ab.	n.r.	
Schimmelbusch	1894	NB	M	pr.	n.r.	
Wetzel	1895	SB	M	pr.	pr.	
Pupovac	1896	9 wks.	M	n.r.	pr.	Metastasis in lymph nodes
Swoboda	1896	8 da.	F	pr.	pr	
Bostroen	1896	NB	n.r.	n.r.	pr.	
Dentler	1902	Fetus 8 mos.	n.r.	n.r.	pr.	Was called cystadeno-enchondrosarcoma
Carter	1903	1 mo.	F	n.r.	n.r.	
Wiesinger	1904	14 da.	n.r.	n.r.	n.r.	
Poult	1905	2 mos.	M	pr.	pr.	
Flesch and Winternitz	1905	a. 8 mos.	F	pr.	pr.	
		b. 2 mos.	F	pr.	n.r.	
Herb	1906	4 da.	M	pr.	pr.	
Lurje	1908	53 yrs.	F	pr.	pr.	Sarcoma with metastasis in lung
Niosi	1908	6 mos.	n.r.	n.r.	pr.	
Hunziker	1909	SB	F	pr.	pr.	
Kimura Kuniza	1910	NB	F	ab.	pr.	
Schoenberg	1911	NB	M	ab.	pr.	
Russell and Kennedy	1913	n.r.	F	pr.	pr.	
Ehlers	1914	9 wks.	M	pr.	pr.	
Dorner	1915	NB	n.r.	pr.	pr.	
Van Rey	1916	n.r.	n.r.	n.r.	pr.	
Fritsche	1920	41 yrs.	F	pr.	n.r.	Sarcomatous structures
Hadda	1921	n.r.	M	pr.	pr.	
Koerner	1922	2 da.	M	n.r.	pr.	
Hördemann	1925	6 wks.	F	pr.	pr.	
Tammann	1925	NB	F	pr.	pr.	
Custer	1926	3 da.	F	pr.	pr.	
Lecène and Mouchet	1928	a. 7 yrs.	F	pr.	pr.	
		b. 2 yrs.	M	pr.	pr.	

Abbreviations: NB = newborn; SB = stillborn; da. = days; wks. = weeks; mos. = months; yr. = year; M = male; F = female; pr. = present; ab. = absent; n.r. = no reference.

cases and shows whether or not the teratoma contains brain tissue. All cases hitherto reported occurred in white individuals.

Hördemann, without giving references, has stated that thirty-one cases of teratoma of the neck had been reported up to 1925. Since Hördemann's publication four more cases have been reported,

yet we have found only thirty-one cases reported which, as far as we can judge, are to be considered true teratoma of the neck. All teratomas of the neck hitherto reported occurred in Europeans, and, with the exception of Herb's case, have been reported in European literature. The material for Herb's report in the American literature was obtained in a hospital in Prague.

Table II shows the cases of tumors of the neck which are not proved teratomas but which, by some authors, have been included as teratoma of the neck.

TABLE II

Author	Year of publication	Remarks
Gilles	1852	Cysts, and cross-striated muscle.
Boucher	1868	Cysts, cartilage, cross-striated muscle.
Tanahill	1871	Cartilage, bone.
Houel	1876	Cysts, cartilage.
Vonwiller	1881	Chondroma mixtum.
Tansini	1888	Glands, cartilage, cross-striated muscle.
Burghagen	1888	Follicles with colloid, cartilage.
Billig	1892	Cysts, cartilage, bone, glandular structures.
Recklinghausen	1893	Enchondroma, glandular structures, cysts.

According to the literature the teratoma of this region seems especially characterized by the presence of brain tissue. Hess was the first to lay stress upon this point. Almost every case reported since 1895 shows glia tissue or ganglion cells as a predominating feature of teratoma in this region.

CASE REPORT

A colored woman was admitted to the hospital with the diagnosis of toxemia of pregnancy. The delivery did not prove difficult but the child was born dead. The child, the subject of this report, was a well developed, mature, still-born, colored girl. She measured 47 cm. in length and weighed 4 kg. In the region of the neck there was a large asymmetrical mass extending across the neck on both sides of the midline, and reaching the angle of the mandible on the left side. The mass measured 3 by 5 by 11 cm. in diameter and was of varying consistency. In parts, the tissue was very soft, almost fluctuant, but in other portions it was very firm and nodular. The firm portions measured about 0.5 to 1.5 cm. in diameter in cross-

section. The skin over the mass was easily removable. The sternocleidomastoid muscles extended over it on both sides. Section showed it to be encapsulated, the capsule containing many hyperemic vessels. The tumor was situated just above the trachea, which showed no signs of compression. A careful search failed to reveal any evidence of the presence of thyroid gland, although the neoplasm was supplied by branches of the superior thyroid arteries.

On section, the fresh surface of the bulk of the neoplasm was grayish white and mucoid in appearance. The tissue was very soft and diffluent, and the capsule retracted. A few portions were reddish gray and of firmer consistency, while other more or less well circumscribed nodules were of a very firm, bony consistency. Other parts showed several cysts which were filled with a clear, pale yellow liquid. These cysts measured 0.5 to 1.5 cm. in diameter and had smooth, thin walls.

With the exception of complete atelectasis of both lungs, the remainder of the organs showed no pathological changes.

Blocks were taken from various parts of the tumor, hardened in 10 per cent formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin and by the Van Gieson method. Mallory's phosphotungstic acid hematoxylin stain was used to demonstrate glia fibers and Bielschowsky's method was applied for identification of nerve fibers.

Most of the sections showed areas consisting of a network of fine fibers which were well stained with Mallory's phosphotungstic acid stain. Between the fibers two types of cells were seen. The smaller cells were deeply stained, showed hardly any cytoplasm, and somewhat resembled lymphocytes. The larger cells had a more or less centrally situated nucleus with distinctly recognizable chromatin network and nucleolus. By the use of the eosin-methylene blue stain it was shown that the cytoplasm contained coarse granules or groups of granules, presumably Nissl bodies. Some of these cells showed protoplasmatic elongations. Blood vessels were abundant in these regions. In short, this type of tissue is identical with brain tissue described recently by Custer in such tumors. The morphology of the large cells, the presence of granules in the cytoplasm and the protoplasmatic processes indicate that these cells are ganglion cells.

One section showed in a circumscribed area a very dark brown pigment which surrounded a small lumen. Poult, Hunziker, Custer

and others suggest that similar pigment found in their cases might possibly be choroidal in origin.

In other fields connective tissue fibers were demonstrable which in some portions were spread apart by a mucoid material containing stellate cells. Some of the sections showed many transversely striated muscle fibers. Most of these muscle fibers contained a centrally situated nucleus but nuclei in the periphery of the sacroplasm and nuclei situated half way between the periphery and centrum of the sacroplasm of the muscle fibers were noted too. Other sections showed a large number of smooth muscle fibers. Young hyaline cartilage with very many nuclei were present in some fields and in only a few areas was well developed bone demonstrable. In some of the sections there was fat tissue.

In a great number of sections cysts were found. The majority of these cysts were lined by one layer of high cylindrical cells, but some were lined by squamous cells. A few cysts showed a very abrupt transition from this type of lining cylindrical cells to squamous cells. Some of the cysts had a papilliferous proliferation of the lining cells into the lumen. Transition from this type of lining cells to squamous cells was also demonstrable. Some of the cysts were surrounded by bands of smooth muscle fibers. Only a few sections contained small acini with empty lumina lined by one or two layers of cuboidal cells. There is no definite indication to warrant the assumption that these acini were parts of thyroid structures.

Several sections showed what appeared to be blood islands. The cells were principally mononuclears but of variable character. In one area they were associated with elongated endothelial cells. Stained with eosin-methylene blue, they were small cells with dense nuclei and scanty, faintly stained, non-granular acidophilic cytoplasm, apparently nucleated erythroyctes. Lymphoid types were characteristic. Larger cells showed a coarsely vesicular indented nucleus. In some of these the cytoplasm was filled with eosinophilic granules of uniform size. In others the cytoplasm was basophilic but not granular. The blood in blood vessels was normal in all respects.

Hair follicles, sebaceous and sweat glands were present in a few slides.

Summarizing the histological findings, the following tissues were found:

1. Brain tissue, which is the predominating part of the tumor, and ganglion cells.
2. Cysts lined by squamous cells; hair follicles; sebaceous and sweat glands.
3. Connective tissue.
4. Smooth and cross-striated muscle fibers.
5. Hyaline cartilage and a small amount of bone.
6. Fat tissue.
7. Blood islands (?).
8. Cysts lined by cylindrical cells; papilliferous cysts. Cysts lined by cylindrical cells with abrupt transition into squamous cells.

Discussion

Our tumor is a true teratoma showing derivatives of the three germinal layers. As in other cases, the predominating part is brain tissue. The location of the tumor, the vascular supply, and finally the absence of the thyroid gland suggest at first that we are dealing with a teratoma of the thyroid. But it is impossible to say whether the tumor arose close to the thyroid gland and merely occupies its place, after atrophy and final disappearance of the gland, or whether the tumor is a teratoma of the thyroid itself. Although most teratomas of the neck show at least parts of the thyroid gland, and ours does not, yet we are rather inclined to believe that our tumor is not a teratoma of the thyroid itself but originated in the neighborhood of this gland.

The age of our patient corresponds with the others reported. Most of the cases reported are in still-born or newborn infants. Only a few are in children several months old. There are only two cases on record of teratoma of the neck in adults. The first one is the case of Lurje, who found a teratoma of the neck in a 53 year-old woman. This tumor showed sarcomatous changes and produced metastasis in the lungs. The second case reported by Fritsche, is one of a malignant teratoma of the region of the thyroid. These tumors correspond histologically with the teratoma of infancy, save for the sarcomatous changes. It is interesting that the only two cases of teratoma of the neck in adults on record showed malignant changes.

All the teratoma of this region so far reported were found in the white race. This is the first case in which such a tumor is reported in a negro.

As to the genesis of the teratoma of our case, our study does not contribute any definite facts. Without going into details of the origin of teratoma, we shall mention only the more recent hypothesis of Budde, which best seems to explain the origin of our tumor. According to Budde, teratomas originate from complexes of cells arising from the symmetrically fusing blastopore, the matrix of the cellular material of the three germinal layers. Since the blastopore extends through the entire length of the "Anlage," Budde believes that this hypothesis serves to provide a uniform monogerminal autochthonous derivation of teratoma of the various regions. According to the degree of differentiation he classifies teratomas into embryomorphous and embryoid teratoma. The embryomorphous is the so-called inclusio-fetalis which he suggests should be called "inclusio-fetiformis." The embryoid type is characterized by a disorderly arrangement of derivatives of the three germinal layers.

Our tumor, according to Budde, is an embryoid teratoma.

Askanazy classifies teratomas into embryonal forms with tissues which are younger than those of the host, and into coetaneous forms in which the tissue is of the same relative age. Our tumor shows well differentiated glia fibers, cross-striated muscle fibers, etc. It therefore should be classified as coetaneous teratoma.

Summary

The literature of teratoma of the neck is reviewed and a new case reported. This is a case of a teratoma of the neck in the region of the thyroid in a still-born colored female. The main constituent of this tumor is brain tissue but derivatives of the three germinal layers are present. For the first time groups of cells resembling blood islands are described in this tumor. Even though the location suggests the thyroid gland as the origin it is more likely that the tumor arose in the neighborhood of this gland. The tumor is classified as an embryoid teratoma according to Budde, and as a coetaneous teratoma according to Askanazy.

Note: I am indebted to Professor H. T. Karsner for his valuable suggestions and for his aid in taking the photomicrographs.

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DESCRIPTION OF PLATES

PLATE 63

FIG. 1. Gross appearance of the body and the teratoma.

FIG. 2. Bone. Hematoxylin-eosin stain. × 115.

FIG. 3. Transversely striated muscle. Hematoxylin-eosin stain. × 600.

FIG. 4. Abrupt transition of two types of epithelial cells. Hematoxylin-eosin stain. × 270.



1



2



3



4

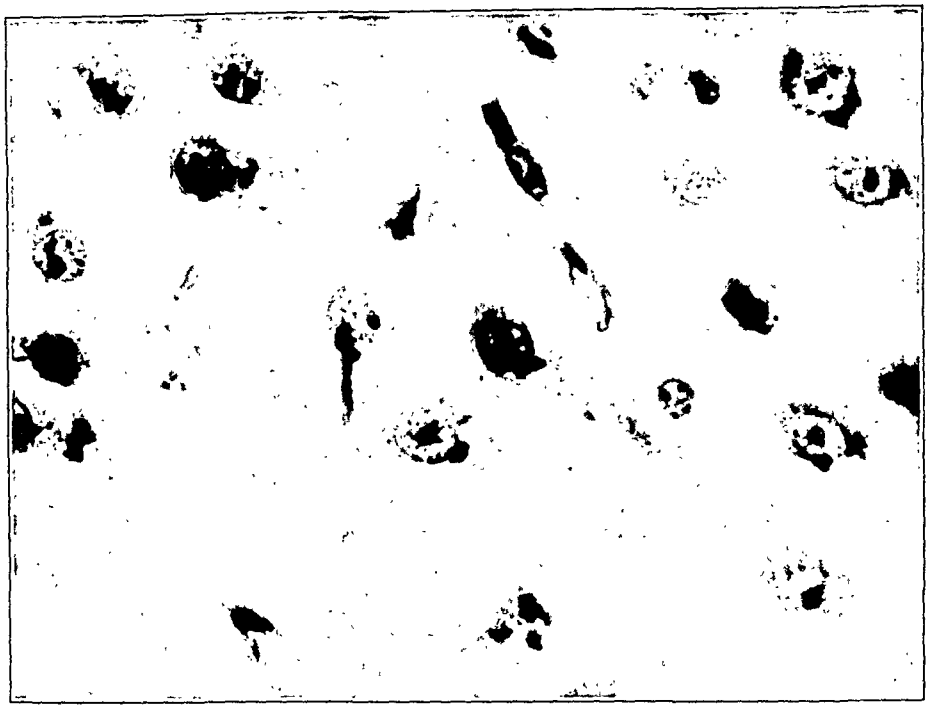
Saphir

Teratoma of Neck

PLATE 64

FIG. 5. Cells resembling ganglion cells. Eosin-methylene blue stain. × 765.

FIG. 6. Groups of cells resembling blood islands. Eosin-methylene blue stain. × 685.



5



6

THE VESSEL CANALS IN NORMAL AND PATHOLOGICAL BONE*

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Blood and lymphatic vessels, if the latter are present in bone, extend through the bone substance in canals. The form and arrangement of these canals changes as the bone is transformed from the coarse-fibered, reticular bone of the embryo and infant, to the fine-fibered, lamellar bone.[1] The transformation in man is completed by about the fourth year and Heuler,[2] studying cross-sections of the human humerus and femur, found that by the eleventh year the adult form is reached and is maintained from then with little change.

CANALS OF FETAL BONE

To trace the origin of the canals of fetal bone, one must go back to the earliest stages of membrane and cartilage bone formation. On studying the ossification of the cartilaginous anlage of a tubular bone, thin laminae of bone are seen underneath the periosteum surrounding the middle of the diaphysis. With further development, the number and size of the periosteally formed trabeculae increase. The newly formed cortex of periosteal bone grows partly by the formation of additional osseous trabeculae on the surface, and partly by the enlargement of the trabeculae already formed. Vessels from the periosteum grow in between the trabeculae, and run toward the marrow cavity. These vessels are directed obliquely longitudinally and the spaces between the periosteal trabeculae constitute the primary haversian spaces, which are the first canals of fetal bone. These haversian spaces are rarely circular in cross-section, being generally somewhat flattened transversely. They communicate with the marrow cavity. The blood vessels contained within these canals may be seen extending from the periosteum into the marrow cavity (Figs. 1 and 2).

In coarse-fibered embryonal and postembryonal bone, the vascular spaces in the cortex are rather large. Their size and shape at this time vary with the part of the bone examined. In a tubular

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bone the spaces of the cortex are larger the nearer they are to the epiphyseal cartilage plate, and in the middle of the shaft of the diaphysis, the spaces nearer the periosteum are larger (Fig. 3).

The bone present between these vascular spaces is coarse-fibered or primary bone, for a description of which the reader is referred to another paper.[1] In the course of development, many of these primary canals under the periosteum become confluent through resorption of their walls and become longer, wider and more irregular. Shortly before and after birth, osteoblasts start to deposit lamellae of fine-fibered bone on the coarse-fibered walls of some of these spaces, which reduces their size considerably.

In embryo or early infancy, canals are present in the cortex which connect the haversian canals with each other and with the marrow cavity. Many of these connecting canals have irregular walls and therefore have been designated by some authors as Volkmann's canals. But this designation is incorrect.

The canals in the epiphyses are not related to the true canals of fetal or adult bone but are mentioned for the sake of completeness. These cartilage canals carry vessels for the nutrition of the cartilage of the epiphyses. They are present in the cartilaginous epiphyses of the larger tubular bones by the third month. This is long before the appearance of ossification centers in them. By the seventh month all the larger epiphyseal cartilages show a rich network of cartilage canals. The general arrangement of the cartilage canals corresponds in a great degree with the arrangement of the marrow spaces of the finished bone that replaces the epiphysis. Most of the cartilage canals enter the cartilage nearly vertical to the surface and run inward, generally toward the site of the future bone nucleus. When the epiphysis is replaced by bone, these canals disappear.

Canals of Adult Bone

With the continued postnatal growth of bone and the transformation of fetal bone into adult bone, the entire canal system of the cortex is reconstructed many times before the adult form is attained. In adult bone, the system of canals consists of the haversian canals, which are the canals of the haversian systems, the canals of the ground lamellae, the communicating canals which connect the haversian systems, and the Volkmann's canals, few of which are seen in normal bone.

Haversian Canals: These canals run longitudinally, as a rule, and are best studied in the cortex of long bones. They are distinguished by the concentric lamellae which surround them. They contain the haversian vessels and together with the concentric lamellae, form the haversian systems. The larger haversian canals may also contain some lymphoid marrow. They may be grossly demonstrated by breaking across the shaft of a long bone near its middle, and examining it with a magnifying glass. Numerous little apertures are then visible on the broken surface, which are the openings of these passages running in the compacta (Fig. 4). In man the canals are from 25 to 125 microns in diameter, but larger ones are also seen. The average size is about 50 microns. In normal bone the widest canals are those nearest the medullary cavity. The diameter of these canals is less in the rat, guinea pig, rabbit and dog than in man.

While these canals run longitudinally, they do not run vertically for more than short distances, soon deviating from the straight line. The canals, as seen in a longitudinal section or ground disc of the cortex, appear as a continuous anastomosing and ramifying network. These canals may extend for one or more millimeters before giving off lateral branches, which may come off almost at right angles, obliquely, or may arch to communicate with an adjacent haversian canal. Sometimes one lateral canal is given off, while at other times several closely placed lateral canals come off from the haversian canal. Between two main lateral branches the diameter of the haversian canal is usually uniform, but after giving off a lateral branch, the diameter may be reduced or increased. The haversian canal may run from a few millimeters to a centimeter or more and then terminate by communicating with an adjacent haversian canal, or it may end in the form of a communicating canal (Fig. 5). The communicating canals between the haversian canals have been wrongly referred to by many as Volkmann's canals. With a few exceptions, these canals are to be viewed as true branches of the haversian canals, and as such they have been formed by the deposition of lamellar bone about preformed vessels which are branches of the vessels within the haversian canals. The lamellae of the communicating canals are generally rounded upwards against the vessel or, turning upward, accompany the vessel within the communicating canal for a short distance as its special lamellae (Fig. 6).

In normal bone there are a few communicating canals of the type described by Volkmann. These canals will be discussed later.

The haversian canals of the compacta become fewer as we proceed towards the extremities of a tubular bone. Beneath the articular cartilage at the upper or lower end of a bone, a longitudinal section discloses a number of haversian canals in cross-section, which means that in this region the canals have changed their direction and run transversely to the long diameter of the bone.

The haversian canals near the surface of the bone communicate with the canals of the ground lamellae which open on the external surface of the bone, and the innermost canals lead into the medullary cavity. In longitudinal sections of bone we have been unable to find more than an occasional haversian canal from the cortex entering the spongy trabeculae.

Some of the larger spongy trabeculae contain haversian canals. A longitudinal section through spongy bone illustrates that most of these canals run transversely to the long axis of the bone. These canals of the spongy trabeculae connect with the marrow spaces by means of short, narrow communicating canals, which are cut through their long axis in a longitudinal section of the bone (Fig. 7).

Canals of the Ground Lamellae: The canals in the ground lamellae vary strikingly, depending upon the bone examined. In certain bones, the inner and outer ground lamellae are very few in number and therefore few canals are present. In animals like the rat and guinea pig, where the ground lamellae are developed to a great degree, a relatively large number of such canals is seen. The canals of the external ground lamellae, when present, are a means of connecting the haversian canals with the periosteal vessels, and the vessels of the internal ground lamellae connect the haversian vessels with the marrow cavity. The canals in the ground lamellae are of two kinds, running either circumferentially or radially across the ground lamellae. These canals are not surrounded by concentric lamellae and they are not very long. Their walls are fairly smooth.

Those canals that run circumferentially are spaces produced by the separation of two ground lamellae. They are narrow, and small vessels are inserted in them. The ground lamellae bordering the canal, without changing their direction, carry the vessel along between them, expanding to conform with the changes in the caliber of the vessel. These are the less important canals. In resorptive

processes in bone they become prominent. I believe that these canals are formed by flattening down of the original haversian spaces on the surface of the bone and the deposition of additional periosteal ground lamellae on them (Fig. 8).

The canals of the other type cross the ground lamellae radially. They appear to be perforating the ground lamellae, but actually they do not, for the ground lamellae were deposited about them. Their length depends on the thickness of the ground lamellae, which varies in species and in individual bones (Fig. 9).

Schwalbe,[3] when he described the canals of the ground lamellae, believed that they were Volkmann's canals and in the textbooks of histology they are designated as such, but this designation is incorrect. These canals communicate with the haversian canals, some open on the surface of the bone and some into the marrow cavity.

The canals of the ground lamellae may be obliterated by the deposition of bone around them, as takes place particularly in those that run circumferentially. The lumen may be enlarged by resorption, in which case lamellae are deposited around the vessel and a typical haversian canal and typical haversian system may then be formed.

Volkmann's Canals: Volkmann[4] in 1862 described numerous vessel canals in the cortex of diseased bone, which differed from normal haversian canals in size, arrangement and vessel content. The number of these canals varied from a few to many, depending upon the bone. These canals had irregular toothed borders, were narrow, but of varying caliber. They were not surrounded by concentric lamellae, but broke through the lamellae surrounding the existing blood vessels. Their course was irregular, being generally horizontal but sometimes diagonal or longitudinal, and sometimes they extended snake-like through the entire thickness of the cortex. These canals have come to be known as Volkmann's canals.

Under the name of haversian spaces, Tomes and De Morgan[5] in 1853 described the same structures in normal bone, the descriptions being almost exactly like those of Volkmann. After Volkmann's original paper, he and Ebner[6] noted the presence of such canals in normal bone. Volkmann first described these canals in a case of osteomyelitis of a metatarsal bone of an old man. The haversian canals of the bone were considerably widened, appearing like irregu-

lar marrow spaces, and contained enlarged vessels and considerable connective tissue. Connecting the enlarged haversian canals were numerous newly formed Volkmann's canals, which traversed the compacta in tangential and radial direction.

We studied these canals in osteoporosis and osteomyelitis. In its earliest form, a Volkmann canal appears as a short, irregular, fine crack in the ground substance, radiating from the haversian canal, and several of them may be seen coming off from one haversian canal. The appearance of the thread-like cracks in the ground substance is associated with the loss of calcium along the path of the crack. The crack rapidly enlarges and grows in length, and gradually increases in width, beginning at the base which is at the haversian canal. An elongated, triangular passage is thus produced. The borders of the canal at this stage are irregular and toothed, and the canal becomes vascularized, but the vessel is preceded, as Pommer [7] pointed out, by some connective tissue, which seems to play a part in opening the crack. As the Volkmann canal grows wider, the toothed contour recedes, and spaces approximately as wide as a normal haversian canal appear (Figs. 10, 11 and 12).

The distinguishing difference between a haversian and a Volkmann canal is that the former results from a concentric deposition of bone lamellae about a preëxisting blood vessel; the latter results from a canalization of fully formed bone by new vessels which break through the existing lamellae tangentially, obliquely, or at right angles to the long axis of the bone.

Soon after Volkmann's descriptions appeared, these canals were observed by other pathologic-anatomists, and controversies arose concerning their mode of origin. Volkmann believed that they resulted from the disintegration of the bone ground substance and that bone lacunae and canaliculi rarely played a part in their production. Occasionally lacunae might be seen opening on a canal, but this happened only when a lacuna lay in its path.

Rindfleisch [8] derived Volkmann's canals from the anastomoses of enlarged bone lacunae and his conclusions were sustained by Soloweitschik,[9] Lossen,[10] and Thierfelder.[11] Kassowitz [12] and Heitzman [13] believed that they resulted from enlargement of canaliculi. These views of the origin of the canals have been abandoned.

In 1885 Pommer observed these canals in osteomalacic and rachitic bones and interpreted true Volkmann's canals as due to the

perforation of formed bone by protoplasmic masses, containing a few nuclei, which grow into the bone from the haversian canal and resorb it. The canal is finally occupied by a blood vessel. Pommer at that time agreed with Volkmann and Ebner that all such canals are newly formed canals, but his explanation of the method of their formation was his own. Recently Pommer[14] and his student, Müller,[15] again emphasized the distinctions which Pommer had previously made between true and false Volkmann's canals. They consider a canal a true Volkmann canal if a new vascular channel, having a funnel-like head and containing a protoplasmic mass, grows through fully formed bone. Unless a canal shows these last two qualities, it is not a true Volkmann or perforating canal, even if its course is irregular and its borders are toothed. They state that most of the canals in bone designated as Volkmann canals, are false Volkmann canals, that is, they are not secondarily formed canals in bone.

Confusion in the Literature Concerning Volkmann's Canals: It would not be necessary to discuss these canals in such detail if the literature were not so confused as to which canals in normal lamellar bone are Volkmann's canals, and as to how these canals are formed. The question also arises as to whether certain canals in fetal bone, designated as Volkmann's canals, should be included under this group, since the original descriptions concerned lamellar bone only.

Let us inquire into the existence of such canals in mature normal lamellar bone. Soon after Volkmann's paper appeared, Schwalbe described the canals of the ground lamellae, and misnamed them Volkmann's canals. As has been stated, he considered these canals as resulting from the deposition of bone around preëxisting vessels. Koelliker[16] agreed with Schwalbe concerning the mode of origin of these canals and also designated them as Volkmann's or perforating canals. In textbooks of histology, the cross canals of the ground lamellae are incorrectly called Volkmann's canals, and Schwalbe and Koelliker are responsible for this erroneous terminology. These canals have none of the characteristics of those which Volkmann described, and which bear his name.

In 1911 Axhausen[17] published a paper dealing with the Volkmann's canals but he studied only the anastomoses which connect the haversian canals of the middle compacta. These canals run transversely or diagonally to the long axis of the haversian canals.

According to him these canals arise by the deposition of bone around preëxisting vessels during bone formation, and generally the only difference between them and haversian canals, is that bone is deposited around the haversian canals concentrically. He believed that these communicating canals were Volkmann's canals. He described three types of canals. In the first type the canals have ragged bone edges. In the second type, the lamellae, as they are deposited about the preformed vessels, are rounded upward against the vessel. In the third type a few of the lamellae, turning upward, accompany the vessels a certain distance, as their special lamellae. Under the heading of haversian canals, I described these communicating canals and pointed out that Types 2 and 3 were an intimate part of the haversian canal system. These communicating canals cannot be termed Volkmann canals. Only the canals of Type 1 are true Volkmann canals. Weidenreich,[18] too, believes that only the canals of Type 1 result from resorption of bone by new-formed vessels. If this were not so, lamellae would have been deposited about the vessels of Type 1 as they were around the vessels of Types 2 and 3.

Recently Zawisch-Ossenitz,[19], working under Schaffer, studied the formation of the communicating canals mostly in fetal bone, and the bones of infants up to two years, but also in some bones of adults. Irrespective of the type of bone, she says that a vessel canal must be regarded as a Volkmann canal when its course is vertical or diagonal to the direction of the lamellae, and when its borders are jagged, with bone lacunae opening on them. Pommer claims that the fetal and postnatal material which she used was not suitable to prove conclusively that all Volkmann canals are due to a vascularization of the bone by new vessels. In her material new vascularization was constantly taking place, and everyone agrees that in such bone, new vessels are constantly perforating the bone. The canals she described in fetal bone cannot be designated Volkmann's canals, for the latter were described in lamellar bone only.

What Should be Included in the Term Volkmann Canals? Volkmann originally reported on the formation of numerous new vessel canals in bone in cases of inflammatory osteoporosis, caries and osteitis, and the presence of a few such canals in normal bone. If the term Volkmann canal is used exclusively to designate the structure described by him, then his original conception, that the canals

result from the canalization of adult bone by newly formed vessels, is correct. As soon as we use the term to designate such canals as Schwalbe described in ground lamellae, or Axhausen in the middle portion of the compacta, or Schaffer and his co-workers in fetal bone and the bone of the newborn, we are applying the same name to different structures. In normal bone the canals (other than the haversian canals and the canals of the ground lamellae) which have resulted from the deposition of bone around preformed vessels, and thus are surrounded by lamellae, should be termed communicating vessel canals. These include most of the canals which join the haversian canals. This type predominates. The canals which have resulted from the resorption of formed bone by newly formed vessels, exemplified by those which have ragged borders and no lamellae, are the only canals which correspond with those originally described by Volkmann, and may be called Volkmann's canals. In normal lamellar bone they are very few in number.

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DESCRIPTION OF PLATES

PLATE 65

FIG. 1. Part of a longitudinal section of the diaphysis of the tibia of a 3-month human fetus. On the right the periosteum is seen, underneath which are several periosteal trabeculae. Between two trabeculae, an obliquely longitudinal haversian space containing a vessel is seen extending from the periosteum to the marrow cavity. Paraffin section, decalcified in Müller's solution and stained with eosin and methylene blue.

FIG. 2. A cross-section through the upper end of the diaphysis of a 6-month human fetus showing the flattened character of the haversian spaces in cross-section. Paraffin section, decalcified in 5 per cent nitric acid and stained with hematoxylin and eosin.

FIG. 3. Part of a cross-section of the diaphysis of a long bone of a 12-week infant, showing the periosteum above, with the subjacent large haversian spaces and the deeper haversian canals, some of which are already surrounded by a few concentric lamellae. Unstained frozen section decalcified in von Ebner's solution.

FIG. 4. Diagrammatic representation of a longitudinal view of a bone, showing both compacta and spongiosa. The cortex on the right shows the outer ground lamellae, with some Sharpey fibers penetrating them. The haversian systems are illustrated and the longitudinally directed haversian canals connected by the communicating canals are shown. After Braus.



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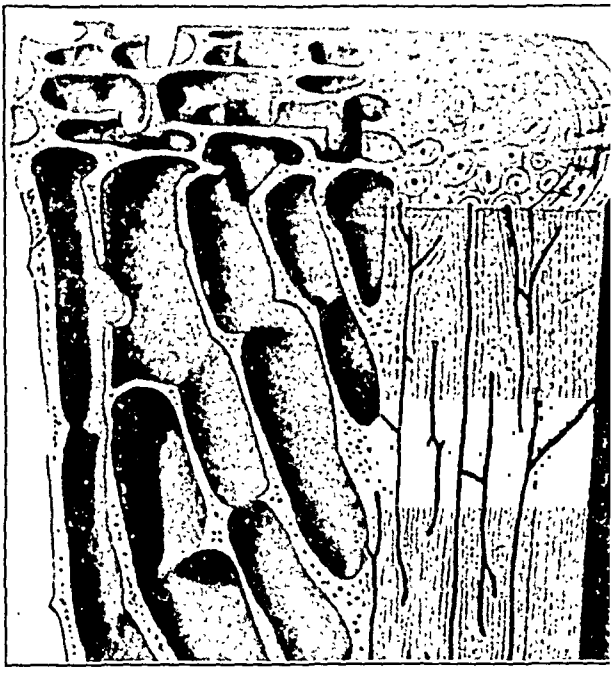


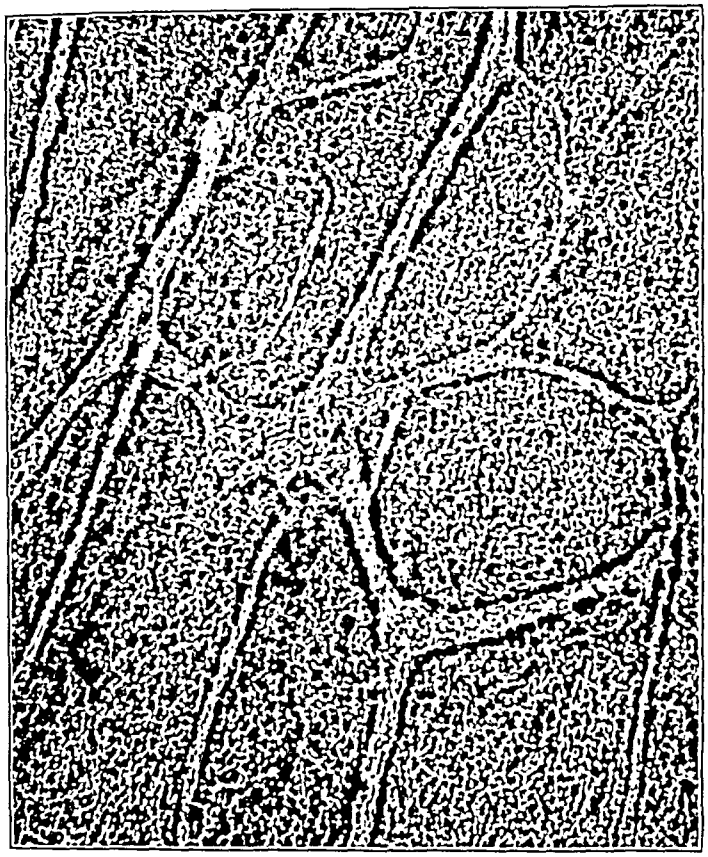
PLATE 66

FIG. 5. A longitudinal section of cortical bone showing the anastomosing and branching haversian canals. The communicating canals between the haversian canals are demonstrated. Unstained ground disc.

FIG. 6. Part of a cross-section of the cortex showing the haversian canals, and in the upper portion of the picture an haversian canal anastomosing with two adjacent haversian canals by means of two communicating canals. Unstained ground disc mounted in balsam.

FIG. 7. Thick, spongy trabeculae from a longitudinal section of a short tubular bone of an adult, showing some haversian canals in cross-section. The canals that connect them with the marrow spaces are cut through their longitudinal diameter.

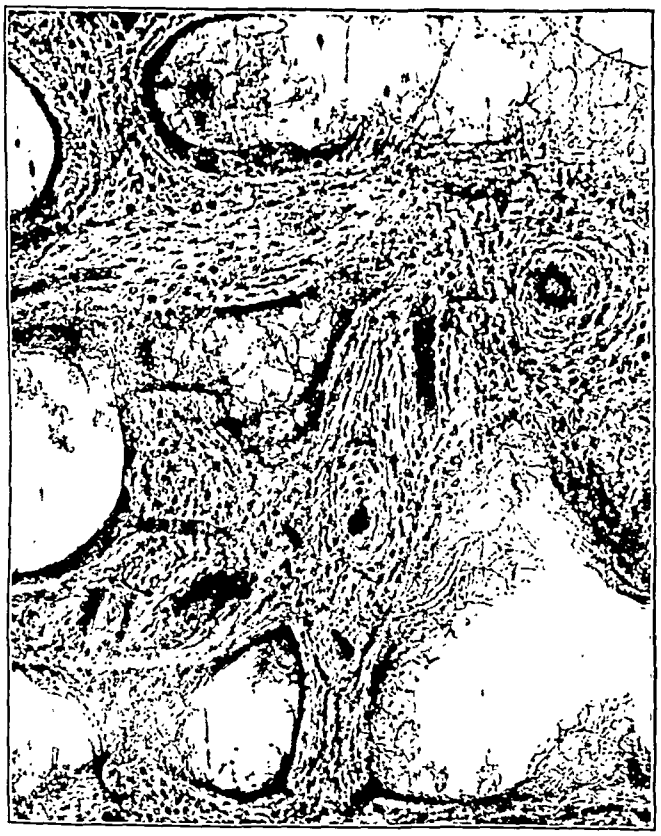
FIG. 8. Section of cortex of a long bone of a dog showing a circumferential canal on the right in the ground lamellae, which is very much widened because the animal was suffering from osteoporosis. On the left, the haversian systems are seen. Unstained frozen section.



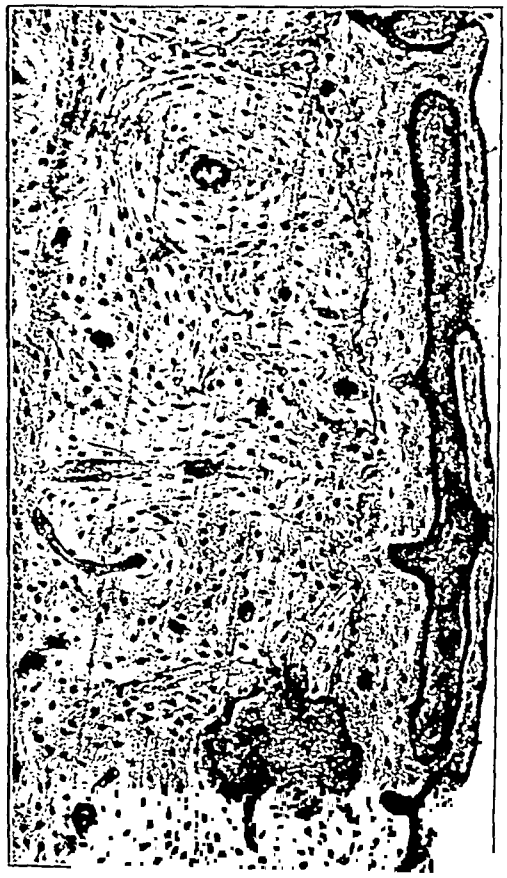
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Jaffe

Vessel Canals in Bone

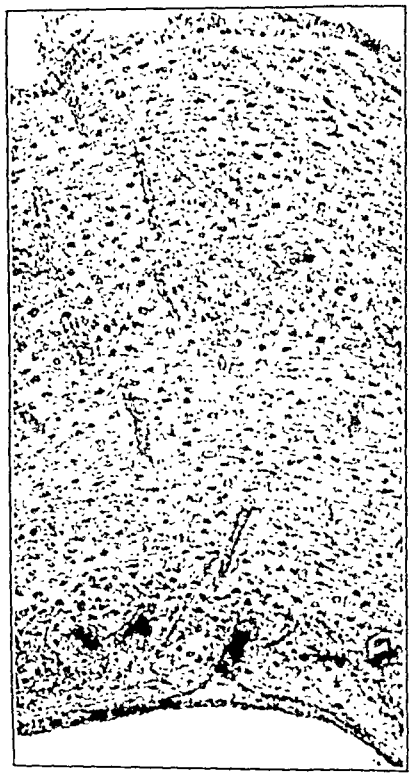
Plate 67

Fig. 9. A cross-section through the tibia of a rat. The compacta is composed almost entirely of ground lamellae and numerous radially directed canals of the ground lamellae are seen. Above, one canal is seen entering from the periosteum.

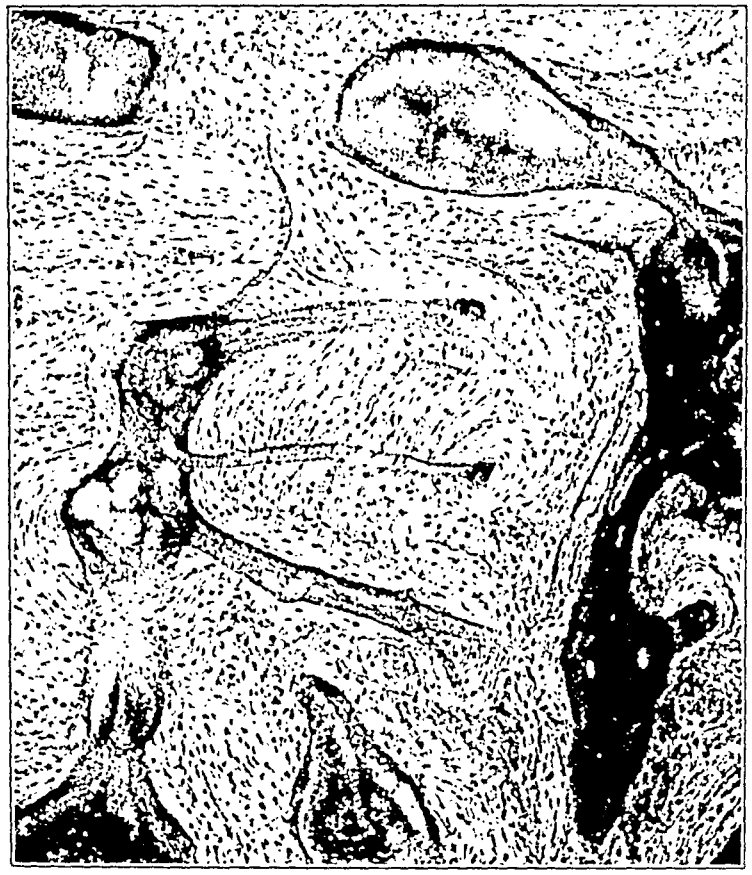
Fig. 10. Cross-section of human adult cortical bone from a case of osteomyelitis, showing enlarged haversian canals from one of which several transverse, newly formed, perforating canals of Volkmann are seen, some of which join other haversian canals. Unstained frozen section.

Fig. 11. Cross-section of the tibia of a dog suffering from osteoporosis. A toothed Volkmann canal is seen connecting two enlarged haversian canals. Unstained frozen section.

Fig. 12. Cross-section of the cortex of human bone from a case of osteomyelitis showing enlarged haversian canals. Two communicating canals are seen in the middle of the picture with the lamellae rounded up against them. They correspond to the canals of Types 2 and 3 as described by Axhausen. Unstained frozen section.



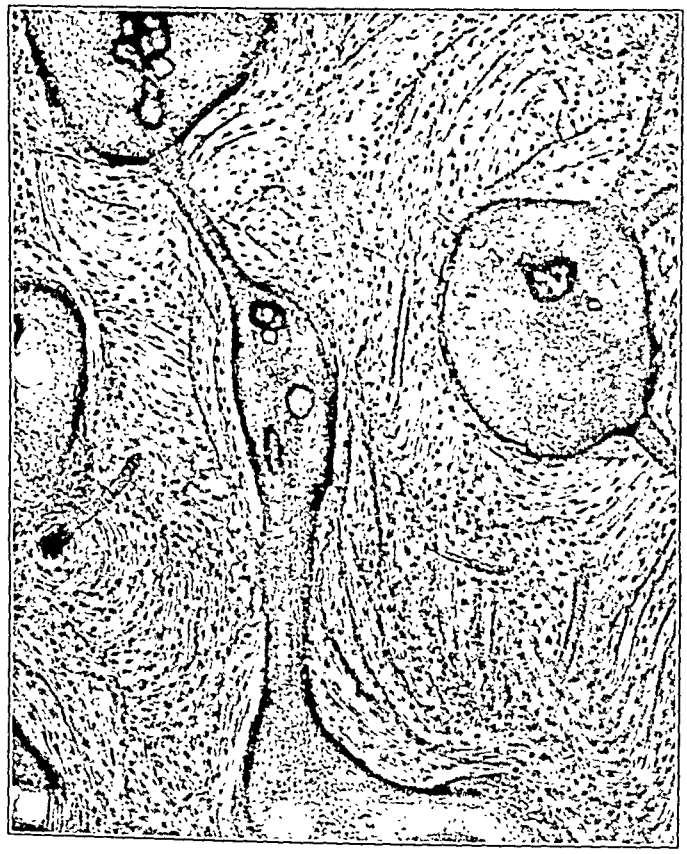
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Jaffe

Vessel Canals in Bone

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ELECTRIC SHOCK *

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The English, American, German, and French literature contains many isolated reports and abstracts of accidents caused by electricity. Great emphasis is placed on the bizarre effects and playful vagaries of electrical current, less is said about treatment and least about prevention.

LITERATURE

No attempt will be made to give a complete review of the literature dealing with electric shock; that is already voluminous and has been clearly described in a recent paper by Jaffé,[1] who in addition gives an excellent bibliography.

The macroscopic and microscopic observations on the nervous system in death from electric shock vary greatly. Some authors found no changes, others, lesions only when large amounts of electricity passed through the body for a long period of time. Some described small perivascular hemorrhages especially in the medulla oblongata and floor of the fourth ventricle, while others reported marked edema of the brain.

Spitzka [2] in his study of the brain of electrocuted animals found no apparent change in the nerve cells, but described areas of disruption and destruction with capillary hemorrhages. In the pons, medulla and cord curious circular areas were encountered with rarefied centers and peripheral zones of condensation, which he suggested resulted from the electrolytic liberation of gas in perivascular spaces.

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Werner [3] in his article dealing with electrocution as a judicial homicidal agent described the brain at autopsy as being very hot and recorded a temperature in the brain of 145° F. Jaffé feels that this excessive heat offers a sufficient explanation for the vacuolization seen within the brain.

Corradi [4] found chromatolysis of ganglion cells in the nervous system of dogs killed by electricity. Mott and Schuster,[5] in addition to perivascular hemorrhages and chromatolysis of ganglion cells in cortex and medulla, described a peculiar intracellular and intranuclear network. Capogrossi [6] also observed marked variation and destruction of the Nissl bodies. Kawamura [7] described shrinkage and hyaline changes in the nerve cells, and fragmentation of the tigroid bodies within the medulla. Gubler [8] reported a vacuolization of the nerve cells and Jellinek [9] destruction of the cells with extrusion of the nuclei.

The histologic changes in the peripheral nerves have been studied by Bolognesi [10] and Pietrusky;[11] the former found swelling and twisting of the axones, and breaking down of the nerve sheaths, while Pietrusky described a fusing together of the nuclei of the sheaths of Schwann.

Report of Experimental Work

A rather small series of animals, namely, one dog and one cat, and twenty guinea pigs, were used in the study of the histologic changes following electrocution.

These animals were divided into two groups; Group I including the dog and the cat, in which death followed immediately after a single shock, and Group II comprising the guinea pigs in which one to three sublethal shocks were given daily over varying periods of time. Of this second group the first animal was shocked on one day only, the second was shocked for two successive days, the third for three days and so on; the animal receiving the greatest number of shocks was shocked every day for nine successive days. Besides varying the number of shocks for each animal, the interval of time that elapsed after the shocking and before the animals were killed and autopsied was also varied; and this ranged from eighteen hours to fourteen days.

The guinea pig was selected because it is a fairly reliable laboratory animal and especially because it is small. When a large animal

is severely shocked the heart stops during the period the current is being applied and then goes into ventricular fibrillation, but seldom, if ever, recovers. The guinea pig heart behaves in much the same manner but if the shock has not been too prolonged the heart recovers its normal rhythm and the animal lives. Briefly, it is considered that the impulse or impulses that are traveling through the myocardium while the ventricle is fibrillating have relatively fewer excitable muscle groups to stimulate in a smaller heart, and consequently fibrillation is of shorter duration and recovery therefore more probable.

In shocking the guinea pigs the current from a six volt battery was passed through a small induction coil, and the strength of the induced current was varied by adjusting the central core. Tissue from a human came to us from a case of legal electrocution, while the dog and cat were obtained immediately after death following electrocution by the Animal Rescue League, and in this case a current of 110 volts was used.

The electrodes that were applied to the guinea pigs were two bared wires; one was passed through and across the mouth, the other was applied to one leg moistened with saline. The Animal Rescue League used a specially built copper cage and collar. In either case excellent contacts were obtained.

Preliminary to shocking the animals sufficient ether was given to quiet them and to permit fastening of the electrodes. Two of four control animals were given ether by inhalation in much larger amounts than the test animals and over a longer period of time.

As soon as the current was passed through the body there was a general contraction of all the muscles, sometimes accompanied by a shriek, and the animal maintained this rigid tonic state as long as the current was applied. The recovery after the shock varied; when the shock was of short duration and not severe, recovery was nearly instantaneous. On the other hand, with more prolonged shocking and with a stronger current, or in animals which had been shocked on several occasions, the recovery was slower, the animals appeared weaker, tremulous and had difficulty in supporting themselves. In several cases they completely relaxed for several minutes, seemed stuporous and assumed unusual catatonic positions. For hours after the shock many of the animals were unusually nervous and excitable, and in two cases they refused ordinary diet but chewed paper con-

tinually for hours and hours. When a fatal shock was given, the animals remained rigid for several seconds after the current was removed, then slowly passed into complete relaxation.

The current probably plays its greatest part in striking the central nervous system and the many altered physiologic signs which become manifest in the different systems appear to be simply visible expressions of extreme stimulation or marked alteration within the central nervous system.

While the current is passing through the body the animal is rigid, the heart stops beating and respirations cease; and eversion of the anus, defecation, micturition and emission occur almost regularly. Occasionally the animals vomit at the onset, and in two there was a prolapse of the rectum which returned as soon as the current was removed. Certain deranged physiologic signs that appear in the nervous system, the heart and blood vessels and in the respiratory system are unusually interesting and bear a more detailed description. Furthermore, to determine whether defecation, micturition, emission and prolapse of the rectum were merely passive reactions secondary to contraction of the abdominal muscles, or result from the stimulation and response of those viscera independently, we repeated the experiments in another group of guinea pigs with the abdominal cavity opened and with still another group in which the animals had received atropine.

THE IMMEDIATE RESPONSE OF THE HEART TO ELECTRIC SHOCK

The heart was examined in each test by auscultation before and immediately after the current was applied, but during the period of shock the sounds of contracting skeletal muscle were so loud it was impossible to follow the cardiac sounds, and consequently it was necessary to observe the heart's action directly.

With the animal lightly under ether and with the heart exposed, an electric shock of five seconds duration was given in the usual manner. As soon as the current was applied the heart contracted strongly and remained in systole except for two to three irregular beats which probably represent vagus escape. During the entire period of shock and for two to three seconds after, the ventricles showed an almost invisible and constant tremor. This was followed by a definite fibrillation of the ventricles which lasted three to five

seconds and this in turn was succeeded by irregular rather forceful contractions which continued for nearly ten seconds. The rhythm eventually became more regular but the rate was slower. This slowing of the heart was seen only in the animals whose hearts had been exposed, whereas in the unoperated animal which recovered after the shock the heart rate was temporarily greatly increased.

This simple experiment was of interest because we were able to follow the heart simultaneously and by auscultation which enabled us to interpret more clearly the irregularities noted in the animals from day to day.

The Immediate Response of the Heart to Electric Shock after Blocking the Vagus with Atropine

In each of these experiments $\frac{1}{100}$ to $\frac{1}{150}$ gr. of atropine dissolved in 5 cc. of sterile water was slowly injected into the leg veins of large healthy guinea pigs. With the animals lightly under ether, they remained sufficiently quiet to permit constant auscultation of the heart. After about ten to fifteen minutes the heart rate had nearly doubled but retained its normal regularity.

In the first of these experiments the heart was not exposed, so we could not follow the beats while the current was passing through the body, but when the current was shut off there was no pause or irregularity and the heart continued its rapid but regular course.

When the heart was exposed and the same experiment repeated, we watched the heart continue beating while the current was passing through the body and we observed no change in rate; neither was there a pause nor irregularity after the current ceased. This was important for it showed that when the vagus was blocked with atropine, an electric shock, which in a control animal was sufficient to stop the heart, produce ventricular fibrillation, irregularity and acceleration, effected no appreciable response in either rate or rhythm.

The Delayed Response of the Heart following Repeated Shocks

In the course of the experiments it was observed that there was a definite change in the heart which became more marked with each additional shock. When the animal was first shocked it usually made

a good recovery and in a minute or two appeared quite normal, but after shocking an animal for several days less current was required to produce the same effect and the recovery was much slower. In such a case the cardiac irregularity was more easily induced and the irregularity and acceleration lasted several times longer than that following the shocking of a normal animal. Furthermore, great caution had to be exercised in shocking animals that had been shocked previously on several occasions lest they suddenly die. Three examples from our series will make this point clearer. One of the guinea pigs died on the fifth day immediately after five shocks; another on the sixth immediately after six shocks. The third, apparently quite well and with a normal cardiac rate and rhythm, had been given seven daily shocks. The heart of the latter pig was being examined preliminary to the eighth shock when the animal exerted a little effort and suddenly died. In the case of the first two we had used less current and for a shorter period than on the preceding day. The manner of death in all three cases was the same. After the heart first stopped the cardiac sounds were never again audible except for three or four irregular and indistinct beats. The animals lay completely relaxed while the respirations continued for two to three minutes in a slow irregular gasping manner.

These observations indicate that as a result of electric shocks there is a profound disturbance in the cardiac mechanism which may be largely obscured by the presence of a normal rate and rhythm.

The Response of the Blood Vessels to Shock

There was a change in the peripheral blood vessels following shock which if duplicated by the blood vessels of the central nervous system might have some consequent bearing on the lesions that later appear in the nerve tissue. By placing the ear of a guinea pig before a light the smaller vessels stood out quite clearly, and these were moderately accentuated if the ear was lightly rubbed. During the shock no apparent change was seen in this rich vascular network, but, about thirty seconds after the current was withdrawn, there was a gradual constriction of the vessels and the ear became abnormally blanched and remained anemic for five to ten minutes. The delayed vasoconstriction resulting from sympathetic stimulation simulates the vascular phenomenon in the condition clinically

spoken of as "surgical shock," and in itself would be sufficient to produce a moderate degree of ischemia with resulting oxygen deficiency in the areas involved.

The Response of the Abdominal Viscera to Shock

We have previously mentioned the observations seen in the normal animal which has neither been operated upon nor received atropine, namely, defecation, micturition and emission and occasionally prolapse of the rectum and vomiting. When the current was applied after the peritoneal cavity had been widely opened to remove the factor of contracting skeletal muscle, we were able to observe the contracting seminal vesicles with the resulting emission, and a slow feeble contraction of the bladder and intestines, although in the latter there was no apparent change in the peristaltic movements.

The pressure within the peritoneal cavity was greatly increased during shock as a result of the contraction of the diaphragm and skeletal muscles; this was well demonstrated when only a small opening was made in the anterior abdominal wall preliminary to an electric shock. As soon as the shock was applied the intestines were forced through the opening.

When the unoperated animal was previously given atropine and then shocked, there was neither defecation, micturition nor emission, even though the abdominal muscles contracted in their usual manner. Similarly, when the peritoneal cavity was opened we observed no variation in peristalsis, in the seminal vesicles or in the bladder.

From these observations in the normal animal, the animal with the peritoneal cavity opened and the animal who had previously received atropine, it would appear that the response of the abdominal viscera was due to the combined effect of contracting skeletal muscle and contraction of the individual viscera themselves.

The Response of the Respiratory Center to Shock

The onset of the shock was accompanied by a deep inspiration, with a powerful contraction of the intercostal muscles and diaphragm. This state was maintained throughout the entire period of shock and respiratory movements did not return for several seconds afterwards when they were shallow, irregular and rather difficult to follow.

PHYSIOLOGIC CHANGES IN THE NEUROMUSCULAR SYSTEM

Probably these were the most interesting of all the varied effects which we observed. We have touched briefly on the rather unusual nervous manifestations, namely, the excitability, nervousness and stupor, and have suggested that the responses seen in the other systems are in reality simply indirect manifestations of an altered nervous system. Now in addition to these there were three outstanding changes which belong specifically to the neuromuscular system alone, namely, weakness, paralysis of the posterior extremities and diminished sensation.

The weakness was usually not seen until after the third or fourth daily shock. The animal became less active, did not move about as quickly, appeared wobbly after a shock, and had difficulty in holding himself up.

Paralysis of both posterior extremities occurred in more than half of the test animals and usually in those who seemed rather feeble. In some cases it came on immediately after the shock; in one it appeared at once, lasted a half hour and disappeared, but again reappeared in twelve hours. Others did not become paralyzed till twenty-four to forty-eight hours had elapsed since their last shock. The paralyzed animals were watched and killed at varying intervals. One that lived fourteen days after the onset of paralysis began to regain some strength and motion in the paralyzed legs. When paralyzed the animals drag their hind feet about and appear to have no control of the bladder or anal sphincters.

If the paralyzed legs were lightly stimulated by touch, pressure, pain or heat there was no visible response. A strong electrical stimulation elicited a contraction of the muscles of the body but the normal reflex arc of the paralyzed legs had been interrupted because the leg muscles showed no response.

The left leg was always used in applying the lower electrode, and even after the first shock the animal would move this less than the other, sometimes dragging it along as though paralyzed. This we attributed to a protective mechanism to relieve the muscular pain.

We shall not attempt at this point to consider the possible pathologic conditions that might account for these neurologic findings as these will be discussed after the gross and microscopic lesions have been described. We have merely enumerated our observations to show the important rôle the nervous system plays in electrocution.

Summary of Physiologic Observations

These manifestations in normal animals, animals that had received atropine, and others with the heart and abdominal and pelvic viscera exposed, are simply expressions of the central nervous system including the craniosacral and sympathetic divisions, and may largely be interpreted on the basis of pathologic histology.

Now it is generally assumed that there are no constant demonstrable histologic lesions in the central nervous system of animals following a single fatal shock in which death is instantaneous. The microscopic study of material from cases of sudden death offers many other examples in which one finds no demonstrable lesions. The classic example is the case of chloroform poisoning; the animal that is killed with a single large dose of chloroform shows no gross or microscopic lesions in the liver, but when an animal receives one or more sublethal doses and is allowed to live twenty-four hours or longer, the degeneration, necrosis and hemorrhage in the centers of the liver lobules are startling.

This principle of giving sublethal amounts of electricity, grading the amounts and allowing the animal to live a sufficient time to permit histologic changes to appear was the method that was followed in this short series of experiments.

Pathologic Report

Complete autopsies were performed on all animals immediately after death. The only positive and constant gross finding in the animals that were given a single fatal shock was marked congestion of the lungs, liver, kidneys, spleen and heart. In the guinea pigs this finding was absent because the animals were bled at the time of death to insure better fixation of the tissues. Hemorrhages were frequently seen in the psoas, iliacus and gluteal muscles, and were usually bilateral. The meninges were injected, and two of the animals had petechial hemorrhages in meninges of both brain and cord. The spinal cord of a normal guinea pig that is autopsied immediately after death is pale, firm and homogeneous throughout. In almost every test animal there were portions of the cord that were distinctly swollen, soft and almost diffluent.

The gross findings in one animal must be described separately because this pig had been given only two shocks. Paralysis suddenly

appeared after the second shock and the animal died in eighteen hours. On examination, hemorrhages were found in the psoas and iliacus muscles, along the anterior surface of the vertebral column, in the erector spinal group of muscles, subdurally along the entire length of the cord, within the cord and also in the brain in the region of the right basal ganglia. This animal, which was the smallest animal we used in the tests, had a definite fracture of the second thoracic vertebra. This gives one an idea of the power of contracting skeletal muscle.

Sections were taken from most of the organs, namely, the heart, lungs, spleen, liver, pancreas, kidney, adrenal, testis, bladder, intestine and skeletal muscle; and of the nervous system from the eye, the cerebrum, cerebellum, pons, medulla, different levels of the cord, pituitary, posterior root ganglia and peripheral nerves.

The tissue, apart from the nervous system, was fixed only in Zenker's solution, neutral formalin and alcohol-formalin and was embedded in celloidin and paraffin. In addition to using the above-named fixatives, sections of the nervous system were also placed in Cajal's formalin-ammonium-bromide solution, Mueller's fluid, and a saturated solution of corrosive sublimate.

In staining the tissues other than those from the nervous system we used hematoxylin and eosin, eosin and methylene blue, Mallory's phosphotungstic acid hematoxylin, Mallory's aniline blue collagen stain, Sudan IV, and osmic acid with and without preliminary treatment with a weak solution of potassium bichromate. For the nervous system we employed hematoxylin and eosin, eosin and methylene blue, Mallory's phosphotungstic acid hematoxylin, osmic acid, Sudan IV, Marchi's method for degenerating myelin, Cajal's gold chloride sublimate method for neuroglia astrocytes and Penfield's second modification of the del Río-Hortega silver carbonate method for oligodendroglia.

Microscopic Report

The pathological histology in the tissues other than the nervous system is disappointing. There is congestion of the heart, spleen, kidneys and adrenals, congestion at the periphery of the lobules of the liver and congestion and petechial hemorrhages in the lungs. The lesions in the skeletal muscles that grossly showed hemorrhages and pigmentation are slight and vary with age. Scattered muscle

fibers are ruptured, the free ends are separated and for a short distance they are contracted, wrinkled, swollen, and homogeneous. Surrounding these lesions extravasated red blood cells lie free in the reticulum. After twenty-four hours polymorphonuclear and endothelial leucocytes are seen within the degenerating and necrotic portions of the muscle fibers, lying free in the stroma, and also in the perivascular tissue. In the later stages after removal of the necrotic portion of muscle fibers, regeneration of muscle occurs, showing the typical multinucleate syncytium resulting from the multiplication of muscle nuclei by direct division and the formation of new cytoplasm. Some areas show degeneration and regeneration occurring simultaneously.

No constant lesion is present in the heart. The amount of fat in the myocardium of both the "test" and "control" pigs is extremely slight and shows little variation. With Sudan IV an occasional muscle fiber is shown to contain minute fat droplets, but this finding is as common in the "control" as in the "test" animals.

The petechial hemorrhages and the capillary injection of the pia arachnoid are verified microscopically and also the hemorrhage extending along the posterior column of the cord of the animal whose vertebral column was broken.

The histologic study of the central nervous system of the animals which received a single fatal shock reveals no constant changes other than capillary injection and occasional petechial hemorrhages.

In the guinea pigs we find definite lesions that vary somewhat in the different animals, depending largely on the severity of the shocks, the number of shocks and the duration of time that elapsed before death. The lesions are best studied in the cord and peripheral nerves including the medullated nerves to the diaphragm and heart, but are also seen within the medulla, in the tracts leading from the pons to the cerebellum and also in the lower portion of the internal capsule. It is the white matter of the cord that is most seriously affected, and in the majority of animals the lesion is greatest in the posterior columns. In some it is more generally distributed throughout all the tracts, in others it seems to affect the fibers lying nearest to the gray matter, while in still others the lesion is distinctly peripheral. In the nerves only scattered fibers are affected.

The earliest change consists in a swelling of both the axis cylinders and myelin. The axis cylinder is enlarged to several times its normal

diameter. In some areas it is disintegrating and in others it has disappeared. The myelin distends its sheath and in the ordinary hematoxylin and eosin preparation has an irregular bluish mucoid appearance. As this swelling progresses the myelin gradually becomes paler till finally it disappears leaving vacuole-like spaces among the fibers.

The picture within the cord differs considerably depending on whether it is viewed from a cross-section or a longitudinal section. In the former the gray matter may show little or no change, whereas the surrounding fiber tracts which comprise the white matter may be riddled with different sized vacuoles. The axis cylinders are not present in some of the larger vacuoles, while in others they are large, irregular and eccentric; even in the comparatively normal areas they are much less uniform in size and contour than one normally finds them. In the longitudinal section the cord has the most bizarre appearance. Axones may be followed only for short distances; they are irregularly swollen and tortuous and appear to taper off at the ends as though disrupted. Myelin, in the later stages, has disappeared leaving irregular vacuoles and long spaces around and among the swollen axones.

In the more advanced lesion where the process has progressed farther, there are foci of marked myelin degeneration heavily infiltrated with phagocytic endothelial leucocytes, scattered polymorphonuclear leucocytes and a few extravasated red blood cells.

The nerves show similar though less marked lesions. Individual fibers are swollen and vacuolated with distention of the neurolemma, while the epineurium is infiltrated with numerous endothelial leucocytes.

The nerve cells from various portions of the normal guinea pig's brain and cord are distinctly different. Moreover, in studying any group of nerve cells whether in the cortex, in the medulla or in the cord, it is found that they differ in size, shape, cytoplasmic staining, in the number of their processes and also in the number, size and position of the Nissl bodies. The nuclei and nucleoli also vary in size, contour, position and staining. Because of these variations it is difficult to diagnose the early degenerative changes in the cells. However, in a few of the areas of degeneration of the cord that extend into the gray matter there is actual necrosis of the nerve cells.

So far we have described the lesion only as it appears when we

used the hematoxylin and eosin, eosin and methylene blue, and phosphotungstic acid stains. When the early lesions are stained with Sudan IV or osmic acid, endothelial leucocytes are seen distended with material resembling myelin, whereas the vacuoles in the cord remain unstained.

In both the cord and nerves of animals which lived one to two weeks after the onset of paralysis, the Marchi method for degenerating myelin produces rather remarkable pictures. There is a much more widespread degeneration of myelin than was expected from an examination of the eosin-methylene blue sections. In cross-section of the cord the degenerated myelin appears throughout the tracts in varyingly sized irregular rings, and also as small and large globules, some of which are in endothelial leucocytes. In a longitudinal section of the nerves, the even uniform distribution of the myelin is replaced by droplets lying in the neurokeratin framework.

Sections of the cord and peripheral nerves were impregnated to bring out both the oligodendroglia and microglia. There is little or no change in the oligodendroglia except in an area of degeneration where they are swollen and rather indistinct. As many as eight to ten perineuronal oligodendroglia are frequently seen surrounding an anterior horn cell in a control cord, and we never found a greater number than this in the test animals. The number of microglia is increased in the white matter, and in areas of myelin dissolution all transitional forms are found between these small cells with delicate branching projections and the large, round, phagocytic, compound granular corpuscles.

The neuroglia astrocytes stain well in both the control and test animals, and though there seems to be an increase of these elements in the posterior columns in the animals with older lesions, yet the number is so variable in different animals and in different levels of the cord that it is doubtful whether there is a beginning true gliosis as early as fourteen days.

In summing up the histologic changes we find that they are confined almost entirely to the nervous system and that they involve primarily the nerve cells, the axones and their myelin sheaths.

DISCUSSION

The question of first importance in electrocution is "what causes death?" Now in speaking of electric currents it is usual to call those over 1,000 volts high tension and those below this, low tension currents, while the importance of either type of current in causing fatal shocks is readily shown in the number of accidental deaths occurring annually. Furthermore, individuals vary greatly in their response to electricity and different portions of the body show wide variation in their electrical resistance. The dry calloused palm of the hand may have a resistance of 2,000,000 ohms, while that of the mucous membrane of the same person may be only 100 ohms.

In our experiments we used very low voltage. When the current was applied the heart ceased beating; this, however, did not occur when a sufficient dose of atropine had been given previously. This suggests that the stopping of the heart results from unusual vagus stimulation. It is stated clinically that in death resulting from shocks of low tension the heart ceases first and respiration continues, while in high tension currents paralysis of the respiratory center is said to precede cessation of the heart beats. Death may be produced readily in guinea pigs using a low tension current, provided the exposure is long enough; with short exposure, however, the heart ceases fibrillation and the normal rhythm returns. In larger animals and man, fibrillation of the ventricle is almost always fatal and so death ensues.

It does not appear to be the heart muscle that is affected by the electric current but rather the entire nervous system. It seems reasonable to suppose that the same factors would apply for either currents of high or low tension and hence the production of apparent cardiac death simply depends on whether or not the heart has been thrown into fibrillation.

There are three possible causes which must be considered in the production of lesions within the nervous system, namely, the electric current, mechanical force and ischemia. We have seen from our experiments the relative importance of these and conclude that the electric current itself is probably the most important single factor.

Death in electric shock may occur at the time of the shock, several minutes later, or days later, or may result from complications resulting from local injury. Occasionally a patient dies suddenly hours

or days after an electric shock. This type of death was seen in the experimental animals in whom it was found that while there were no demonstrable lesions in the myocardium, there were definite lesions in the cord, peripheral nerves and nerves to the heart. Hence, we feel that though a patient may appear in normal health after a shock reasonable precautions should be taken for days and weeks to avoid unnecessary stress and strain.

Summary

1. The literature dealing with the pathologic changes in the nervous system of electrocuted animals is reviewed.

2. The observations in normal animals, animals with exposed viscera and animals under atropine which were receiving electric shock are described in detail.

3. The value of repeated sublethal shocks in obtaining histologic lesions is stressed.

4. The rôle of the nervous system in producing changes in other systems is discussed.

5. The gross and microscopic lesions are described in which it is shown that the lesions are confined almost entirely to the nervous system and skeletal muscle.

6. The discussion deals with physical properties of electrical currents, the resistance of the body to currents, the different types of death from electric shock and emphasis is laid on the importance of prolonged rest in cases of recovery.

Note: I am indebted to Dr. F. B. Mallory for his many suggestions and helpful criticism, and also for the photomicrographs; and to Miss Marion E. Lamb for technical assistance.

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DESCRIPTION OF PLATES

PLATE 68

FIG. 1. Section of cord removed fourteen days after the onset of paralysis and stained by Marchi's method for myelin degeneration. There is marked myelin degeneration in the lateral tracts, while just beneath and partly involving the posterior horn is an area of "softening" in which the degenerated myelin droplets have coalesced; many of them are in phagocytic endothelial leucocytes. × 100.



1

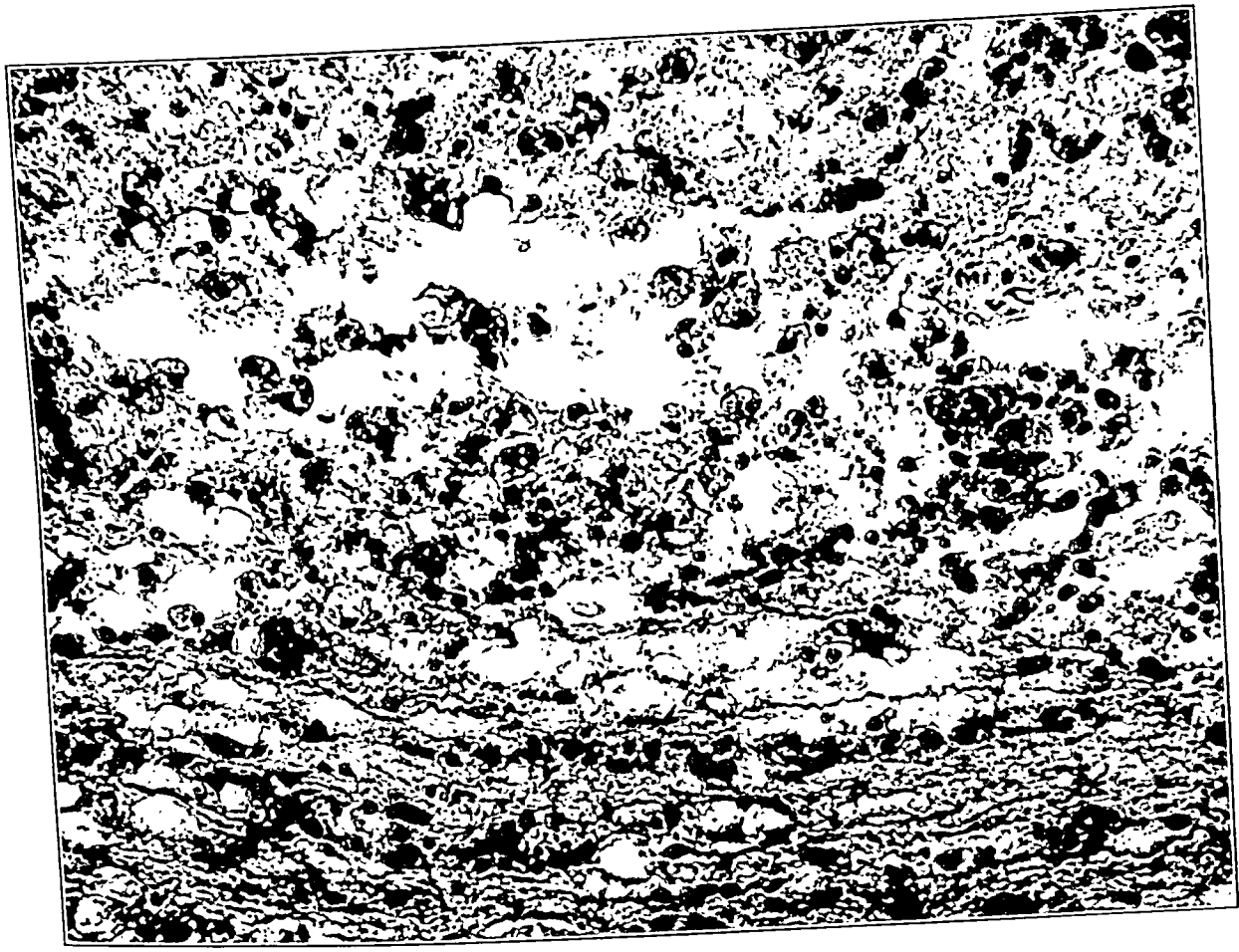
PLATE 69

FIG. 2. Photomicrograph of a longitundinal section of the cord showing in the posterior columns the swollen, tortuous axis cylinders. The clear areas represent swollen myelin or areas from which myelin has entirely disappeared. There is a moderate infiltration of endothelial leucocytes. (Hematoxylin and eosin.) × 300.

FIG. 3. Photomicrograph showing an area of "softening" in the posterior column of the cord. The normal morphology and arrangement of the elements are destroyed. Axis cylinders and myelin have disappeared; the latter is in part phagocyted by endothelial leucocytes. (Hematoxylin and eosin.) × 250.



2

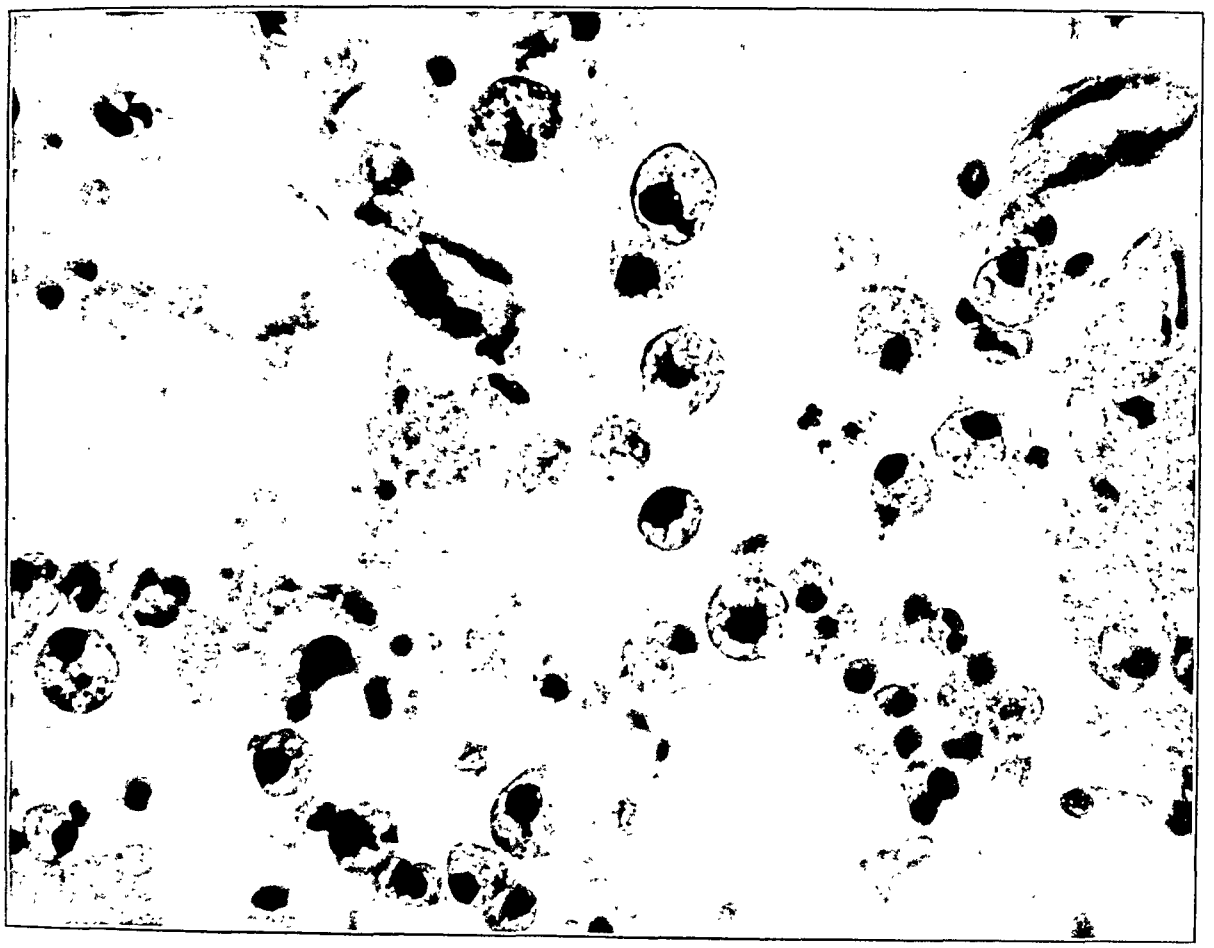


3

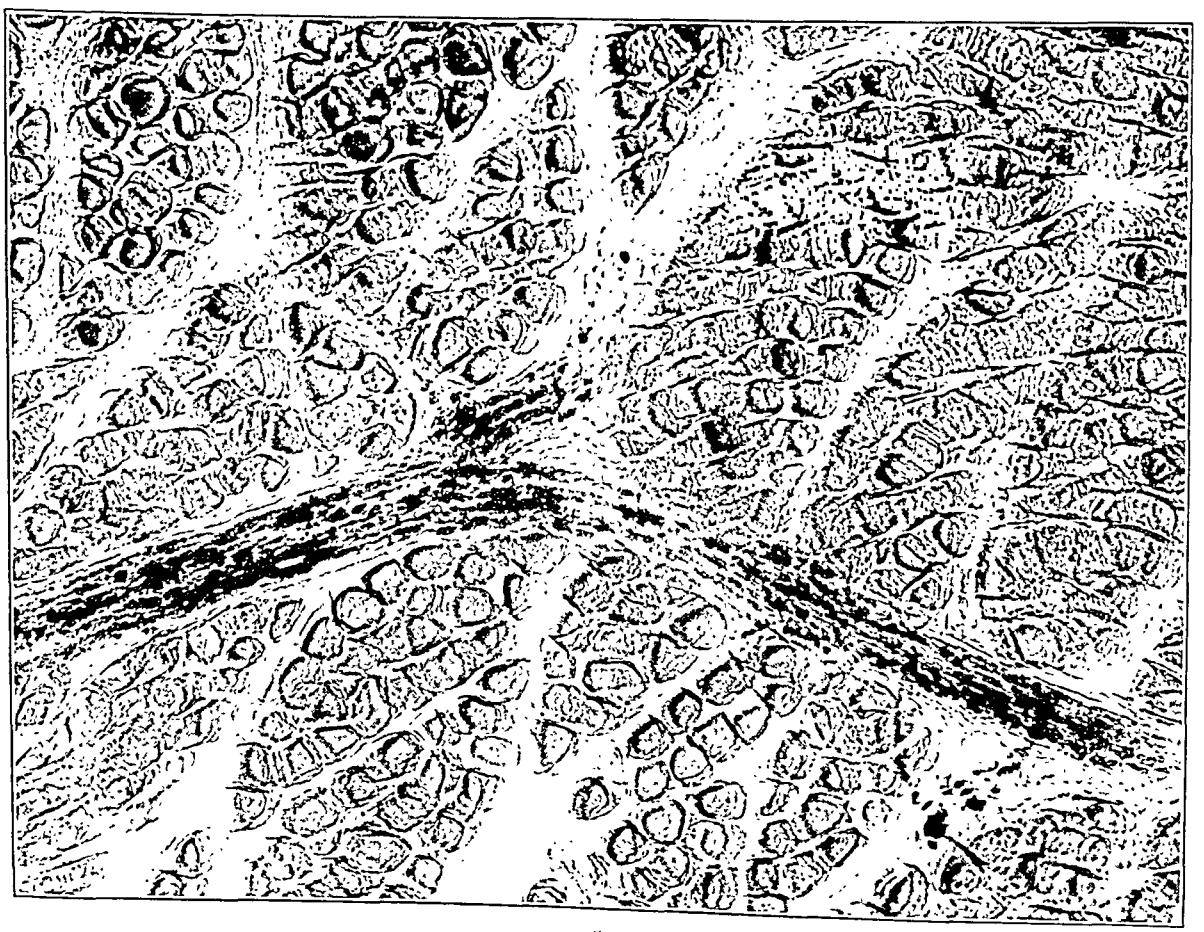
Plate 70

Fig. 4. A higher power photomicrograph from an area of degeneration in the cord, showing endothelial leucocytes many of which are distended with myelin. × 500.

Fig. 5. Longitudinal section of a peripheral nerve traversing bundles of striated muscle. Degenerated myelin droplets of various sizes are held in the neurokeratin framework of the nerve fibers. Marchi stain for degenerated myelin. × 125.



4



5

MacMahon

Electric Shock

A CASE OF MENINGITIS DUE TO ENDOMYCES CAPSULATUS (NEW SPECIES) *

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The purpose of this paper is to report the clinical record and autopsy findings of a fatal case of systemic *Endomyces capsulatus* infection. The organism was isolated from the tissues and cultured. This paper includes a study and description of the morphology, cultural characteristics and animal pathogenicity of the organism.

Fungi may attack any organ or system of the body, the integumentary system being most frequently and the nervous system most rarely affected. Diseases of fungus origin are called mycoses. These may be classified into two groups — internal mycoses and external mycoses or dermatomycoses.

Castellani [1] has stated that in cases of general infections due to fungi like Saccharomyces, Monilia and Endomyces, abscesses containing such fungi may be found in the brain and spinal cord. In this country cases of cerebrospinal-meningitis have been recorded due to *Cryptococcus histolyticus* (synonym *Torula histolyticus*), Stoddard and Cutler,[2] 1916. It has been shown that Torula has a special predilection for the central nervous system and lungs.

Summaries of our knowledge concerning *Torula histolytica* infections in man have been made from time to time since Stoddard and Cutler's monograph was published in 1916. The more recent reports are by Shapiro and Neal [3] in 1925, and Rappaport and Kaplan [4] in 1926. Shapiro and Neal mentioned thirteen cases then reported. They added another case and included a personal communication from Sheppe. This made the total reported cases fifteen up to that time, of which thirteen were infections of the central nervous system and meninges, one of the lung, and one of the muscles of the back. Rappaport and Kaplan reported a systemic torulosis and McKendree and Cornwall [5] in the same year reported an infection of the brain

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and meninges. An account by Hansmann [6] in 1924 of a case of meningo-encephalitis caused by *Torula histolytica* was not mentioned by these writers. About this time Lynch and Rose [7] reported an additional case of meningo-encephalitis due to *Torula histolytica*. Hall, Hirsch and Mock [8] in 1928 added a case of meningo-encephalitis. Wortis and Wightman [9] in 1928 reported another case of *Torula histolytica* meningitis. Hence we have collected a total of eighteen cases in which *Torula histolytica* was the etiologic factor in meningitis.

A case of sporotrichosis meningitis was reported by Hyslop, Neal, Kraus, and Hillman [10] in 1926.

Vialard, Lancelin, and Darleguy [11] in 1925 reported a case of meningo-encephalitis due to Trichomyces in a case of pulmonary tuberculosis.

In 1917 Swift and Bull [12] reported a case of primary blastomycotic meningitis. Since then in 1922 Williams [13] has recorded a case of meningitis which followed a case of pulmonary blastomycosis. Badham [14] has mentioned a case which appeared in Sydney in 1922. Watanabi [15] reviewed a case in which blastomycotic invasion of the tonsils was reported.

Report of Case

Clinical History: J. M. (Hospital No. 568,911), a white, married male, 48 years of age, who was a furrier by trade, entered the Boston City Hospital, August 30, 1928, complaining of continuous frontal and temporal headaches during the past three and a half months.

Family history was negative, and the past history was without interest.

His present illness began five months before entry when he noticed a hard tender slightly movable mass about the size of a hen's egg on the medial surface of the lower third of the left thigh. It gradually subsided during the next four weeks, at the end of which time a similar mass appeared beneath the anterior surface of the neck. A short time after the first mass subsided the patient began to have continuous and almost unbearable frontal and temporal headaches. Two weeks before entry he developed defects in memory, would lose himself on familiar streets, could not remember places and lost track of time. He lost interest in everything and seemed not to understand questions or conversation. He gradually became worse and was brought to the hospital.

Physical Examination: A well developed and emaciated middle-aged white man who was dull mentally and slightly confused. A tender cystic mass about the size of a hen's egg was situated at the level of the thyroid isthmus between the trachea and the anterior border of the left sternocleidomastoid muscle. The chest was symmetrical. Resonance over both lungs was impaired and over the right apex were heard both fine and medium crepitant rales. A small tender cystic mass about the size of a walnut was felt on the medial surface of the lower

third of the left thigh. Temperature, 98.6° F; pulse, 84; respiration, 20; blood pressure, 130 systolic and 80 diastolic.

Neurologic Examination: The pupils were contracted and reacted slowly to light and accommodation. The fundi showed perivascular streaking, venous engorgement and slight pallor of both discs. Bilateral diminution of hearing. The deep reflexes were exaggerated more markedly on the right. The cremasteric and Babinski reflexes were absent. During the examination the patient was very emotional, having fits of crying and laughing.

Laboratory Findings: The urine and blood examinations were negative except for a 5 per cent eosinophilia. The blood Kahn was negative. The spinal fluid showed an initial pressure of 210 mm., a slow fall after abdominal and jugular compression, leucocytes 20, of which 15 were polymorphonuclear and 5 lymphocytes, Wassermann positive and gold sol. 0000022220. The spinal fluid was cultured on blood agar, chocolate agar and Sabouraud's medium, and showed no growth at the end of fourteen days. A smear of this spinal fluid was negative. The second spinal fluid was similar except that the pressure and dynamics were normal and the gold sol. 0001242110. Culture of the spinal fluid on blood agar, chocolate agar and Sabouraud's medium, showed no growth at the end of fourteen days.

September 11, 1928: The cyst in the thyroid region was aspirated, and 30 cc. of thick creamy tenaceous yellow pus was obtained. Smears of this pus contained numerous polymorphonuclear leucocytes and countless numbers of round granular cells with vacuoles and refractile bodies in their cytoplasm. These cells were encapsulated and were one fourth the size of a red blood cell. Numerous budding forms were present. The organism was cultured and identified as *Endomyces capsulatus* (new species). Organisms, which were identical morphologically and culturally with the organisms cultured from the pus from the neck, were found in the pus aspirated from the nodule on the left thigh.

September 16: X-ray report: extensive bilateral pulmonary tuberculosis; skull plates were negative.

After a short stay the patient left the hospital against advice when a biopsy was suggested. His condition was unchanged.

Clinical Diagnoses: Mycotic encephalitis(?); mycotic abscesses of the neck and left thigh; bilateral pulmonary tuberculosis.

October 3, 1928: The patient was readmitted.

Interval History: (Hospital No. 571,024). Since his discharge the patient had become completely disoriented. A week before entry he became incontinent and lethargic.

Physical Examination: The patient was comatose and had mild choking of the discs. He had a stiff neck and bilateral Kernigs. Babinski's sign was positive on the left and questionable on the right. Temperature, 98.6° F; pulse, 80; respiration, 20.

Laboratory Findings: The urine and blood examinations were negative except for a 4 per cent eosinophilia in one smear. Spinal fluid showed an initial pressure of 110 mm., the dynamics were normal, total protein 171 mg., leucocytes 51, of which 62 per cent were lymphocytes, gold sol. 2233443100, chlorides 568 mg. and sugar 36 mg. Culture of the spinal fluid on blood agar, chocolate agar and Sabouraud's medium showed no growth at the end of fourteen days. The second spinal fluid showed chlorides 579 mg., sugar 39 mg., total protein 190 mg., and leucocytes 68. Culture, guinea pig, and mouse inoculations were negative. These animals were killed and autopsied two months after the inoculation.

The patient's condition became worse, his temperature, pulse and respirations rose rapidly, and he died October 31, 1928.

Clinical Diagnoses: Mycotic meningo-encephalitis(?); bilateral pulmonary tuberculosis.

An autopsy (A–28–350) was performed eleven hours postmortem. The tissues were immediately placed in Zenker's fixative and later embedded in paraffin and stained with eosin-methylene blue, phosphotungstic acid hematoxylin, Mallory's aniline blue, Gram Weigert's method and by Ziehl–Neelson's method for tubercle bacilli in paraffin sections.

Autopsy Report

The body is that of a well developed and emaciated middle-aged white man. A red scar 1.5 cm. long is present to the left of the midline on the anterior surface of the neck. A similar scar is present on the medial surface of the lower third of the left thigh. The left femoral lymph nodes are palpable.

Peritoneal Cavity: On the serosal surface of the ileum are two congested areas 2 cm. in diameter, and a third is present on the cecum near the ileocecal valve. In these congested areas are numerous firm gray nodules measuring about 0.1 cm. in diameter. The remainder of the peritoneal cavity is without interest.

Pleura and Lungs: The pleural surfaces are smooth and glistening save for the left apex where there are dense fibrous adhesions joining the visceral and parietal pleural surfaces. Numerous firm irregularly shaped and sized nodular masses are present throughout both lungs involving the upper lobes more than the lower. Fresh section of these firm masses reveals numerous closely approximated firm yellowish white nodules measuring 0.1 cm. in diameter. No cavity formation present. The bronchi and the mediastinal structures are negative.

Heart and Spleen: Without interest.

Gastro-Intestinal Tract: Stomach negative. Two ulcerative lesions in the lower ileum and a third in the cecum near the ileocecal valve are similar in appearance. In the ileum they entirely encircle the bowel and are 1.5 to 2 cm. broad. The margins of the ulcers are ragged, nodular, irregular, thickened and undermined. Their bases are covered with yellow necrotic material. The serous surface is congested, and numerous gray nodules about 0.1 cm. in diameter are present in the subserous tissues.

Liver, Pancreas, Kidneys, Adrenals, Bladder and Genital Organs: Without interest.

Organs of the Neck: The thyroid, trachea, esophagus and larynx are negative. To the left of the midline the subcutaneous tissues are flesh-colored, granular and are studded with numerous firm nodules 0.2 cm. in diameter.

Left Thigh: The subcutaneous tissues beneath the scar on the left thigh are flesh-colored, granular and are studded with numerous firm nodules measuring 0.2 cm. in diameter. The tissue involved is about 2 cm. in diameter.

Head: The scalp and calvarium are negative.

Brain: Weight 1,120 gm. The dura is tense. When opened the hemispheres bulge out and when uncovered present a flattening of the convolutions and an obliteration of the sulci. The cerebrospinal fluid in the subarachnoid space is clear. The lower surface of the cerebellum, pons, medulla and the cranial nerves arising from the brain stem are covered with numerous granular, closely approximated, firm, flesh-colored nodules. No abscesses or nodules are observed in the brain substance. The ependyma is rough and granular. The ventricles are moderately dilated.

Anatomic Diagnoses: Nodular meningitis; granular ependymitis; nodular cellulitis of the neck and left thigh; bilateral pulmonary tuberculosis; tuberculous ulcers, ileum and cecum.

Microscopic Examination

Lung: The normal architecture is obliterated by roughly concentric lesions. Endothelial leucocytes and foreign body giant cells are present in and around central zones of necrosis which are composed of shadowy remains of cells. Acid-fast bacilli are present in the necrotic centers. At the periphery of the lesions is a narrow border of lymphocytes. Conglomerate lesions similar in structure are present. The adjacent alveoli are filled with endothelial leucocytes.

Heart and Spleen: Without interest.

Intestine: An ulcer with an undermined edge extends down to the muscularis. In the base of the ulcer and extending out beneath the undermined edge are lesions similar to those seen in the lung. Acid-fast bacilli are present in the necrotic centers of the lesions.

Liver: Small discrete lesions similar to those seen in the lung and intestine are present.

Pancreas, Kidney, Adrenal, Prostate, Thyroid and Pituitary: Without interest.

Subcutaneous Nodule, Left Thigh: In the subcutaneous tissues there are roughly concentric and conglomerate lesions composed of endothelial leucocytes and one or more foreign body giant cells and surrounded by a narrow border of lymphocytes. A moderate amount of connective tissue surrounds the lesions. In the larger affected areas are foci of necrosis in which are found many polymorphonuclear leucocytes, fewer vacuolated endothelial leucocytes, and an occasional foreign body giant cell.

In the cytoplasm of the endothelial leucocytes and foreign body giant cells are round yeast-like organisms which are surrounded by a clear non-staining capsule. These organisms vary from 0.7 to 2.8 microns and average 1.6 microns in diameter. Within the organisms are round and oval basic-staining bodies resembling nuclei. In the small organisms only one is present, while in the larger ones two and even three have been observed. In sections stained by Mallory's aniline blue the capsule of the organisms stains blue. The organisms are gram positive.

Within the tissues this organism reproduces by budding. By studying the organism in the foreign body giant cells and endothelial leucocytes, all stages of budding have been observed. An early stage is characterized by a slight protoplasmic bulge at the periphery of the organism accompanied by a similar bulge of the capsule. In another cell may be seen a well developed bud which is still joined to the parent cell. A later stage is observed in which a capsular septum separates the daughter from the parent cell. In the last stage the separation is complete, each cell having its own capsule.

Medulla: On the pial surface of the medulla and surrounding the cranial nerves there are roughly concentric and conglomerate lesions composed of endothelial leucocytes and one or more foreign body giant cells and surrounded by a narrow border of lymphocytes. In the larger lesions are areas of necrosis in which are found many polymorphonuclear leucocytes, fewer vacuolated endothelial leucocytes and an occasional foreign body giant cell. In the cytoplasm of the foreign body giant cells and endothelial leucocytes are round yeast-like organisms, surrounded by a clear non-staining capsule. These organisms are similar to those seen in the tissues of the thigh.

Microscopic Diagnoses: Mycotic meningitis; mycotic cellulitis, left thigh; tuberculosis of lungs; tuberculous ulcer, ileum; miliary tuberculosis, liver.

Bacteriologic Study

A nodule removed from the pial surface of the medulla was washed in several changes of sterile saline, ground in a mortar, suspended in saline and cultured on Sabouraud's medium. Colonies of Endomyces were observed on the seventh day. Two mice were inoculated with this suspension. The first mouse died on the sixth day, and the second on the seventh. Gross examination was negative except for multiple abscesses in the kidneys. Microscopic examination was negative except for the kidneys. Large areas in which the normal architecture was destroyed were infiltrated by polymorphonuclear and a few endothelial leucocytes. Endomyces within endothelial leucocytes were present in the abscess and in the adjacent tubules.

B. Coli was cultured from the heart's blood and spleen.

Morphology

Methods: Fleming's weak killing and fixing solution was added directly to the agar tube containing the fungus. After remaining in this solution for about twenty-four hours, the material was washed in running water from twelve to twenty-four hours. After washing, the material was removed from the culture tube, a thin slice of agar containing the fungus on one surface was cut from the agar plug, transferred to commercial hydrogen peroxide (3 per cent) and bleached. This material was then stained either with Delafield's hematoxylin or with Heidenhain's iron alum hematoxylin and counterstained with an aqueous solution of eosin. When the material was satisfactorily stained it was transferred to a 10 per cent aqueous solution of glycerol which was allowed to concentrate by evaporation of the water until an equilibrium was reached. The material was then mounted on slides, gently teased apart as much as possible with fine needles and a cover glass added.

ENDOMYCES CAPSULATUS, REWBRIDGE, DODGE AND AYERS: NEW SPECIES

The mycelium in lesions consists exclusively of budding cells; see Figs. 22, 23 and 24. In culture, when first isolated, and in acid media, the mycelium consists of coarse thin-walled hyphae, 3.5 to 4 microns in diameter, composed of nearly isodiametric cells (Fig. 3). Hyphae, often somewhat contorted, suggest the mycelium of Madurella. On neutral media with protein as the principal source of nitrogen the hyphae are slender, branched, 1.4 to 1.5 microns in diameter with long, mostly uninucleate cells.

Budding cells about 3.5 by 5 microns are abundant on acid media, but absent on neutral media (Fig. 3).

Hypnospores (chlamydospores) terminal on short branches or in chains, develop from mycelium resembling the raquet mycelium of Trichophyton and related genera. The cells of this mycelium are 6.5 to 7 microns in diameter at the swollen end and 2.6 to 3 microns in diameter at the other end (Figs. 15 and 19). In cultures on corn-meal agar, large spherical cells, 11.3 microns in diameter, develop on the ends of short branches. They have very thick walls but their contents seem to be degenerating (Fig. 1).

Conidia are sessile, pyriform, 5.5 by 6.5 microns, developing irregularly along the mycelium but usually near a septum, more abundant on media with protein nitrogen and a neutral reaction (Figs. 14 to 16).

Endospores (*endoconidies* of Vuillemin[16], *globules internes* of Salvat and Fontoynont[17]) are ellipsoidal, 2.6 by 3.2 microns in the hyphae on Sabouraud's dextrose agar (Fig. 12). These spores are formed by the rounding up of the protoplasts into deeply staining, ellipsoidal masses, but no spore wall was observed and their significance is still uncertain. Their appearance suggests that of the hormogonia of the Myxophyceae.

Sexual reproduction when present is heterogamous. A cell of a filament swells to about 11 microns in diameter and fuses with an apparently little differentiated cell (4.8 microns) on a hypha (3.6 microns) which may lie nearby (Figs. 6, 7, 9). Whether this is a case of amphimixis or pseudomixis or whether it is related to the curious sexuality of *Ascobolus carbonarius* reported by Dodge[18] must await a further study of the nuclear divisions preceding and during this

copulation. Since pseudomixis is common among the yeasts it might be expected here.

The details of development between copulation and the formation of the asci have not been sufficiently studied. The asci are terminal on long filaments, eight-spored, subspherical, about 7 by 9 microns. The ascospores are hyaline, spherical, smooth, 2 microns in diameter (Fig. 4). So far, asci have occurred only in cultures on Sabouraud's dextrose agar about nineteen weeks old.

Cultural Characteristics

The original isolation furnished us from this case was cultivated at room temperature (about 20° C). It was transferred to a variety of media roughly ranging from pH 4.5 to 7.2. Since our laboratory is poorly equipped for accurate determination of the concentration of hydrogen ions, in this preliminary work we have been content to accept the statement on the labels of recent batches of dehydrated media from the Digestive Ferments Company and will postpone further detailed consideration of the physiology of this fungus to a later paper.

Since a cursory examination of the original culture showed the general morphology of a Monilia or of some other imperfect yeast, we tried it on a number of acid media which are usually satisfactory for this group. It will be seen, however, that for this organism media with a lower hydrogen ion concentration and more complex nitrogenous compounds were more satisfactory. Since most of our time has been devoted to the morphology of the organism we have not yet attempted to study its growth on slightly alkaline media, but since our best growth occurred on Bacto Nutrient (beef) agar at pH 7.2, it is probable that growth will occur on more alkaline media.

In the following descriptions of colonies the media have been arranged approximately in the order of decreasing concentration of hydrogen ions.

Wort Agar (Bacto pH 4.5): No growth.

Wort Agar: (Approximately the formula published for Bactowort agar with slight increases in amounts of maltose, dextrin and [Bacto] peptone, which would probably cause a slight decrease in concentration of hydrogen ions.) Surface of the colony moist, shining, yeast-like at first, becoming dull or somewhat powdery in age.

Growth slow, reaching less than 1 cm. in diameter in six weeks, elevated, convolute to cerebriform. Mycelium very irregularly branching, septate; cells very short (Fig. 3). This culture after five months resembles most closely the freshly isolated cultures.

Malt Extract Agar (Bacto pH about 4.7): Growth slow, scarcely covering the surface of the tube in nineteen weeks, forming a compact thin felt in the older portion, with only a very sparse or fine powdery surface in the younger portion. Mycelium more filamentous than in wort agar with very few yeast cells.

Sabouraud's Dextrose Agar (Bacto pH 4.74) and Sabouraud's Conservation Agar: Growth more rapid, the colony reaching 2 cm. in diameter in two weeks, white, well developed at the inoculum, surrounded by a loose cottony zone with a dense cottony periphery. Mycelium filamentous, hyaline, branched, composed of clavate cells, 2 to 4.5 microns in short diameters. Hyphal tips swollen, suggestive of the raquet mycelium of Trichophyton. It should be noted that the original cultures on Sabouraud's agar were less definitely filamentous and more yeast-like. It is not clear whether that particular batch of agar was more acid or whether frequent transfers on artificial substrates have gradually adapted the fungus to a changed environment. The writers are inclined to the former alternative in view of the growth on media of higher concentrations of hydrogen ions.

Corn-meal Agar (Bacto pH 5.9): Growth similar to that on Sabouraud's agar but much less developed and much slower, covering less area in eight weeks than it does on Sabouraud's medium in two weeks. Mycelium very slender, cells very long. Thick-walled hypnospores abundant.

Swordfish (Sterilized Muscle of Xiphias Gladius L., pH 6.1): Growth very slow, colony less than 0.7 cm. in diameter in three weeks, more compact than colonies on corn-meal agar, quite elevated. Mycelium slender, cells long, producing abundant conidia, many of which are terminal on short branches, arising at right angles to the hyphae as in Acremonium.

Cooked Egg: (The whites and yolks were beaten together, coagulated, then tubed and sterilized at a pressure of 15 lb. per square inch for twenty minutes; pH unknown.) Growth slow, similar to that on swordfish but conidia much less abundant.

Beef Agar (Bacto Nutrient pH 7.2): Growth rapid, forming a

thick, smooth felt over the whole surface. Mycelium very slender; conidia abundant.

Animal Pathogenicity

Guinea pigs, rabbits and mice were used for study. A culture of *Endomyces capsulatus* on a Sabouraud slant incubated at 37° C for seven days was suspended in 20 cc. of saline. Suspensions made in this way were used in the following animal experiments.

Each one of four guinea pigs was inoculated intraperitoneally with 4 cc. of the above described suspension. At the end of eleven weeks they were healthy and had not lost any weight. They were then killed and autopsied. The four guinea pigs were grossly and microscopically negative. Cultures of the heart's blood on Sabouraud's medium and blood agar showed no growth at the end of seven days.

Each one of four rabbits was inoculated intravenously with 4 cc. of the above-described suspension. At the end of twelve weeks they were healthy and had not lost any weight. They were then killed and autopsied. The four rabbits were grossly and microscopically negative. Cultures of the heart's blood on blood agar and Sabouraud's medium showed no growth at the end of seven days.

Each one of four mice was inoculated intraperitoneally with 0.5 cc. of the above described suspension. These mice developed a progressive infection, became emaciated and died between the third and fourth weeks. The lesions found at autopsy were identical.

Gross Examination: Numerous firm granular nodules measuring 0.2 to 0.3 cm. in diameter were present in the omentum and on the peritoneal surface. Similar granular nodules were observed in the lungs, liver, spleen and kidneys.

Microscopic Examination: In several large areas the normal architecture of the lung is destroyed, and the cavity formed is filled with polymorphonuclear leucocytes, a few endothelial leucocytes and countless numbers of encapsulated Endomycetes. Numerous budding cells are present. Similar lesions are present in the liver, spleen, kidneys and omentum.

Cultures of the heart's blood on blood agar and Sabouraud's medium showed no growth at the end of seven days. Peritoneal nodules were cultured on Sabouraud's medium. Colonies of Endomyces were observed on the seventh day.

Summary of Animal Experiments

1. Mice when inoculated intraperitoneally with *Endomyces capsulatus* develop a progressive infection affecting the peritoneum, lungs, liver, spleen and kidneys, resulting in death at the end of three to four weeks.

2. The organism Endomyces can be cultured from the lesions.

3. The organism was not cultured from the heart's blood of mice killed by this infection.

4. Rabbits after receiving an intravenous inoculation were healthy and had not lost any weight at the end of twelve weeks. They were grossly and microscopically negative.

5. Guinea pigs after receiving an intraperitoneal inoculation were healthy and had not lost any weight at the end of eleven weeks. They were grossly and microscopically negative.

Classification

The Saccharomycetales, to which the organism here studied belongs, are divided into three families: the Dipodascaceae, a primitive family with several features which are suggestive of the higher Phycomycetes; the Endomycetaceae or filamentous yeasts; and the Saccharomycetaceae or true yeasts. Besides these there are a large number of organisms whose life cycles are incompletely known which probably belong to these groups. From a survey of the medical literature dealing with these organisms, the nomenclature appears chaotic and the application of generic names uncertain. It seems best to the writers to consider species having a variable amount of filamentous mycelium, which shows the characteristic budding associated with the yeasts, as belonging to Monilia, and the species with spherical or nearly spherical cells which may adhere in chains but which never form characteristic filamentous mycelium as Cryptococcus. While it is never safe to predict the genus of Ascomycetes to which one of the Fungi Imperfecti will belong when the whole life cycle is known, it is probable that the species of Monilia will belong to Endomyces and those of Cryptococcus to the Saccharomycetaceae.

The Endomycetaceae are usually divided into about a half-a-dozen genera of which only two, Eremascus and Endomyces are at all well known cytologically. Eremascus, found on fruit juices, lacks

the characteristic budding of Endomyces and has regular isogamous reproduction.

Endomyces which grows on a wide variety of substrates shows great variation in its reproduction. The species may be divided into two series, one of which has retained its primitive isogamy and gradually degenerated until the sexual act becomes rare and parthenogamy the rule; the other series starting with quite well defined heterogamy as in the present species has also degenerated towards parthenogamy, so that the end results of the two series are identical or at least so similar that it is difficult to differentiate them.

If we exclude species whose ascogenous stage is unknown, species which have often been included by their authors in Endomyces, very few species have been found in man or other mammals. Some of these as *E. Cruzi* De Mello and Paes, and *E. pulmonalis* Senez were described in journals to which we have not had access. The second, perhaps, belongs in Monilia. While we have not seen the original description of *E. bonaerensis* Greco,[19] the discussion by its author (1916) leads us to place it either along with *Monilia alba* (Queyrat and Laroche [20]) Sartory [21] (1922) from vaginitis (not the organism described under that name by Castellani in 1911), or with *E. Vuillemini* Landrieu, closely related to *Monilia albicans* of thrush and similar infections. During the campaign in Mesopotamia, *E. tropicalis* Castellani and Acton [22], which produced creamy patches on tonsils and uvula that were difficult to remove but left no raw bleeding surfaces and were accompanied by diffuse inflammation of the uvula, of pillars of the fauces and of the posterior pharyngeal wall, was described as having a very curious life cycle. If the description should prove to be correct, it should certainly be removed to a new family if not to a new order of fungi. It certainly has little in common with the organism under consideration.

E. crateriforme Hudelo, Sartory and Montlaur [23] produced an oval, erythematous, scaly lesion in the armpit of a young woman reminding one of dry seborrheic eczema. It was not pathogenic to guinea pigs or rabbits by subcutaneous, intraperitoneal or intravenous injection, although scarification produced non-typical evanescent lesions. It is evident that it was unable to invade the deeper tissues of the body differing markedly from *E. capsulatus*.

Only one true Endomyces, *E. Molardi* Salvat and Fontoynont [17, 24] remains for discussion and separation from our organism. The

former was reported from a lesion on the leg, originally hard nodules which softened into an abscess which was finally cured after about two months by external application of methylene blue. Inoculation in the thigh of a guinea pig produced a local abscess which healed spontaneously. Intraperitoneal injections were fatal in thirty-three to forty-four days with cachexia accompanied by nephritis. The organism was recovered from the blood.

The mycelial filaments are 2 to 6 microns in diameter, somewhat contracted at the septa, and thick-walled. The filaments may end either in a simple cell; a group of short thick-walled, nearly spherical, yeast-like cells, sometimes twice the diameter of the penultimate cell; or by a spherical cell 8 by 22 microns in diameter. The latter are evidently hypnospores and sometimes occur in groups or short chains. The asci and ascospores are produced on glycerol agar or on carrot plugs with glycerol. The asci vary from spherical to ellipsoidal with four, rarely eight spores.

On glycerol agar the growth was rapid, thick, creamy or ivory white, elevated, with small deeper yellow craters in the middle. On Sabouraud glucose agar growth was less abundant, consisting of plateaux slightly raised in the center, creamy white, glistening, then opaque, with fine radiations from the central cone about the fifteenth day. A comparison of *E. Molardi* with our organism shows differences in most points of morphology, culture and pathogenicity, although it is certainly congeneric.

Summary

1. The clinical record and autopsy findings of a case of meningitis due to *Endomyces capsulatus*, n. sp., are reported.

2. The organism was not found in the spinal fluid.

3. The morphologic and cultural characteristics of *Endomyces capsulatus*, n. sp., are described.

4. The organism is pathogenic for mice but not for rabbits or guinea pigs.

Note: We are indebted to Dr. F. B. Mallory and Miss Catherine G. Norton for the illustrations and to Miss Lillian M. Leavitt and Miss Marion E. Lamb for technical assistance.

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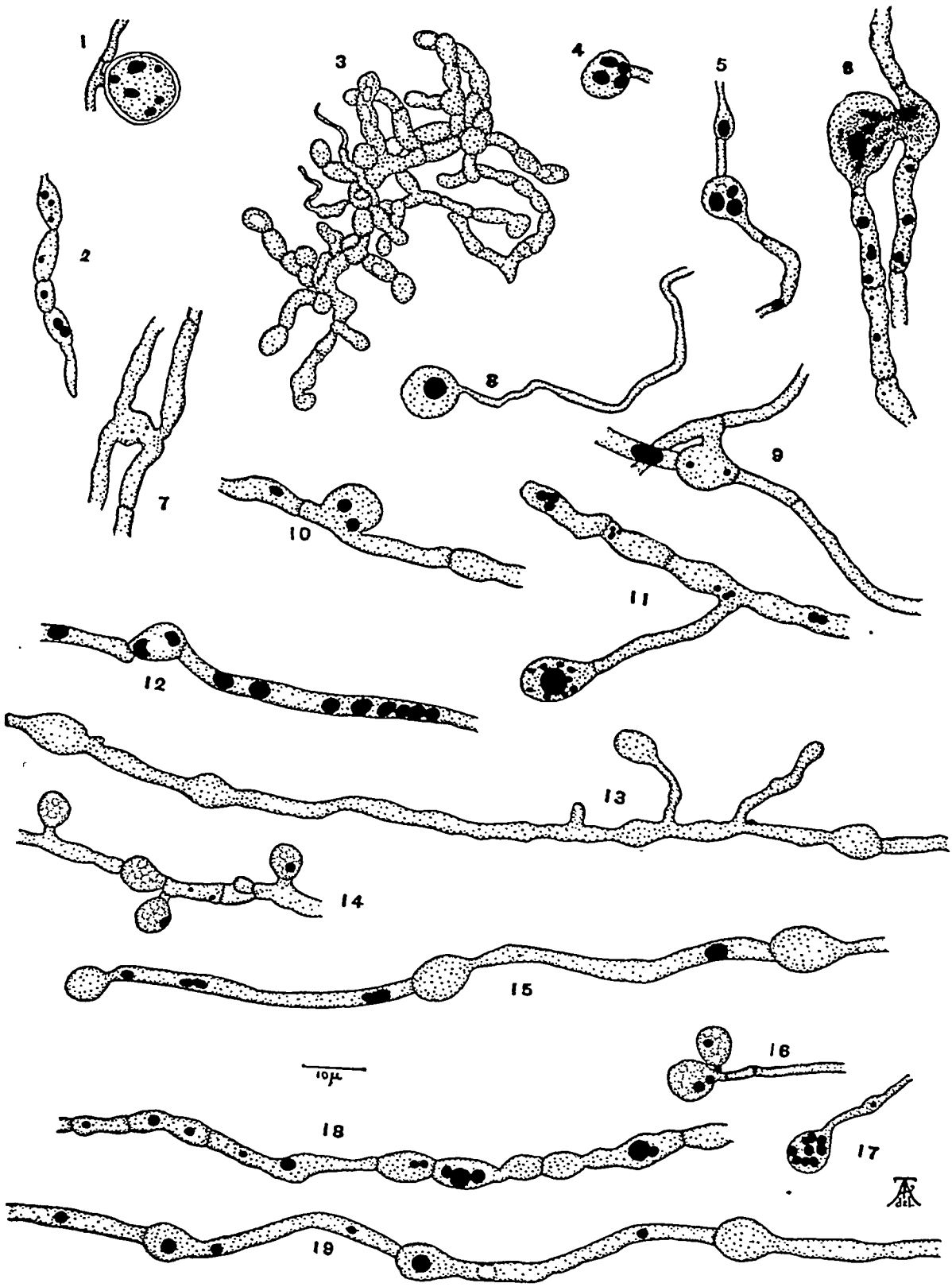
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DESCRIPTION OF PLATES

PLATE 71

The interpretation of some of our figures must await further cytological study: Fig. 1 from corn-meal agar, Fig. 3 from wort agar, Figs. 14 and 16 from malt extract agar, the others from Sabouraud's dextrose agar.

FIG. 1. Large, thick-walled cells produced on corn-meal agar, function not yet known. × 1100.
FIG. 2. Hypha on Sabouraud's dextrose agar, suggestive of budding mycelium. × 1100.
FIG. 3. Yeast-like growth on wort agar. × 1100.
FIG. 4. Ascus showing ascospores. × 1100.
FIG. 5. Raquet mycelium. × 1100.
FIGS. 6 and 7. Heterogamous copulation. × 1100.
FIG. 8. Perhaps young ascus before spore formation, showing fusion nucleus. × 1100.
FIG. 9. Heterogamous copulation. × 1100.
FIG. 10. Perhaps earlier stage before nuclear fusion. × 1100.
FIG. 11. Terminal hypnospore. × 1100.
FIG. 12. Portion of hypha showing endospores. × 1100.
FIG. 13. Type of mycelium. × 1100.
FIG. 14. Conidia. × 1100.
FIG. 15. Type of mycelium. × 1100.
FIG. 16. Conidia. × 1100
FIG. 17. Probably an ascus. × 1100
FIGS. 18 and 19. Types of mycelium. × 1100.



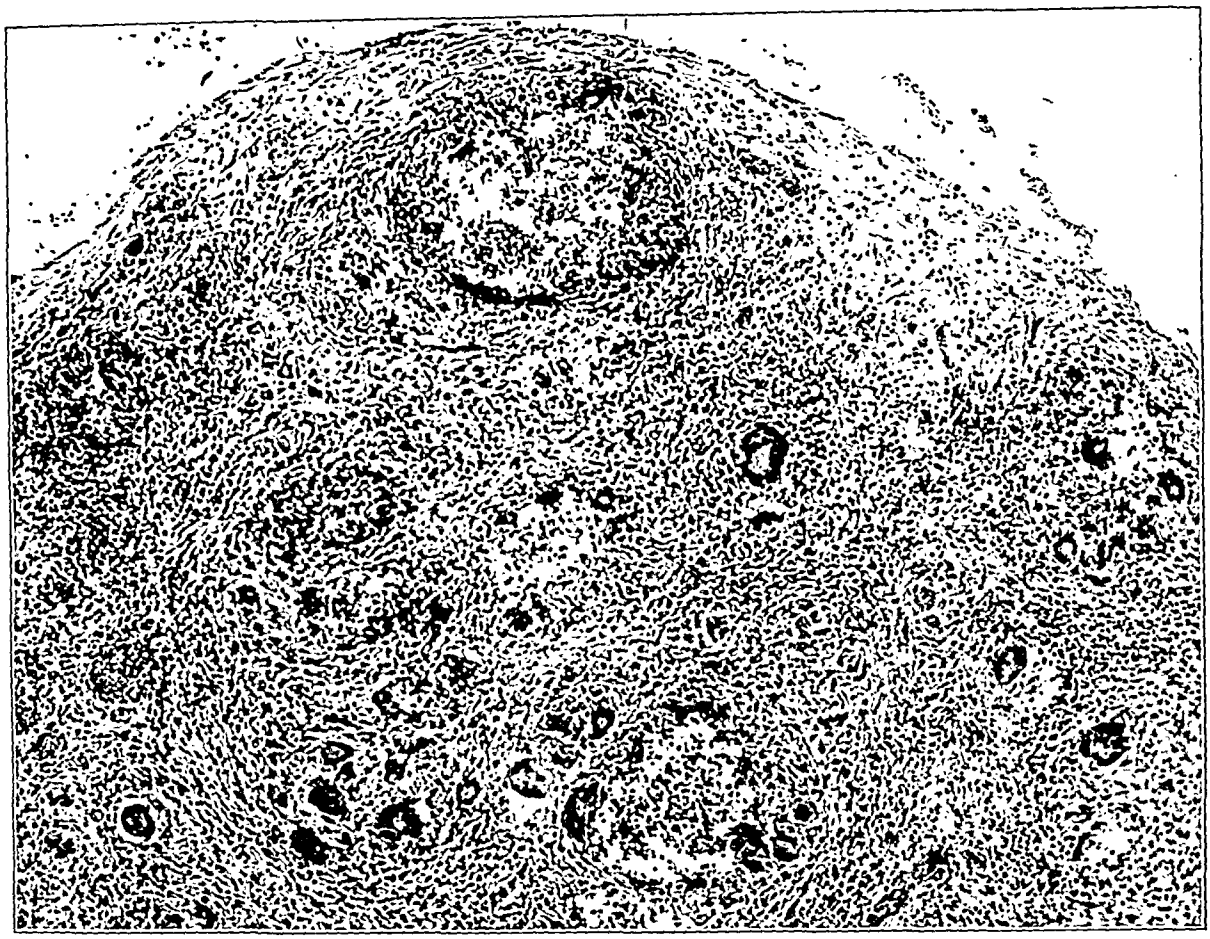
Rewbridge, Dodge and Ayers

Meningitis Due to Endomyces Capsulatus

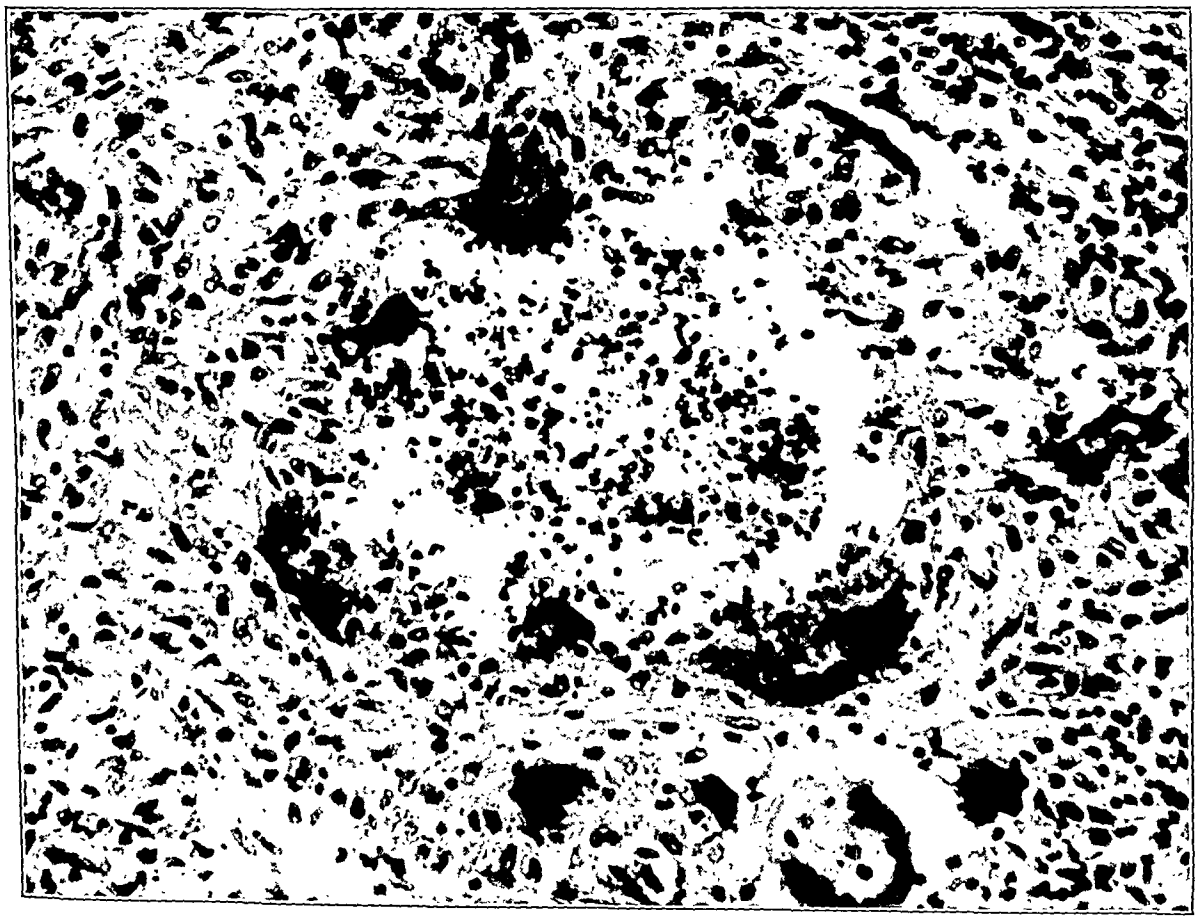
PLATE 72

FIG. 20. Photomicrograph of a section through a pial nodule showing an exudate consisting of foreign body giant cells and endothelial leucocytes. There are several areas of necrosis in which are found many polymorphonuclear leucocytes. × 100.

FIG. 21. Photomicrograph showing an area of necrosis in which are found many polymorphonuclear and fewer endothelial leucocytes. Several foreign body giant cells border the necrotic area. × 300.



20



21

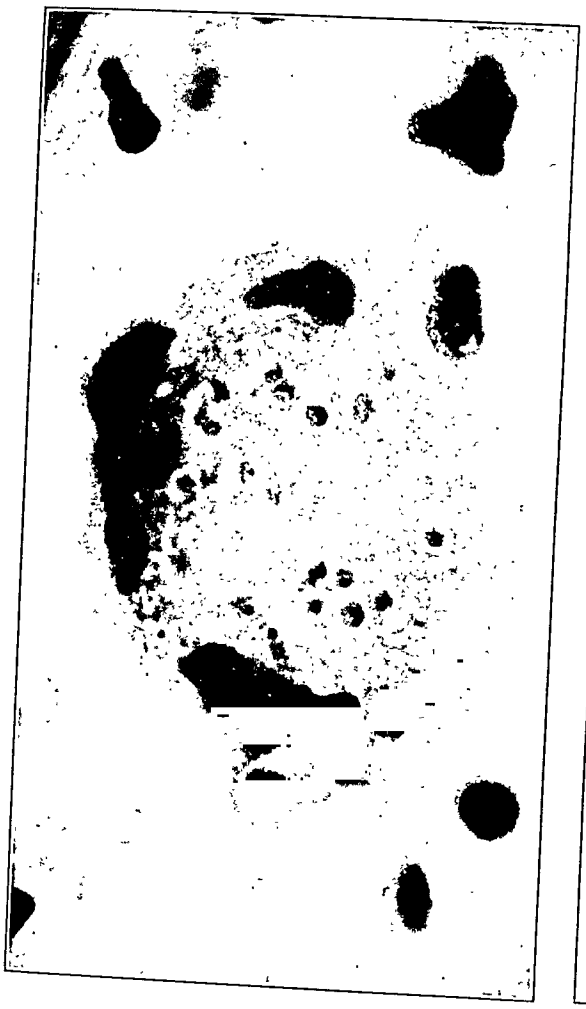
Rewbridge, Dodge and Ayers Meningitis Due to Endomyces Capsulatus

PLATE 73

FIG. 22. In the cytoplasm of the foreign body giant cell are round yeast-like organisms surrounded by a clear non-staining capsule. × 1500.

FIG. 23. Three budding organisms are present in the cytoplasm of the foreign body giant cell. × 1500.

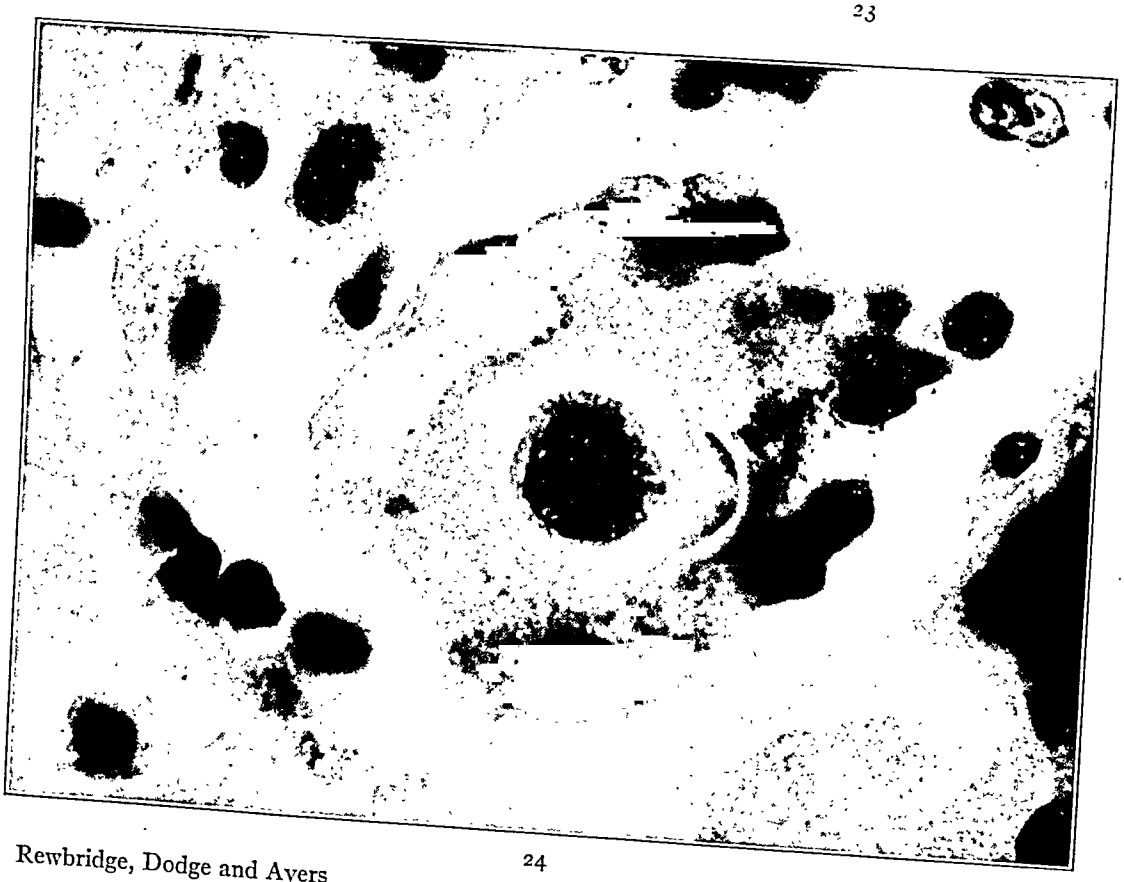
FIG. 24. Photomicrograph showing an early stage of budding which consists of a slight protoplasmic bulge at the periphery of the organism accompanied by a corresponding bulge of the capsule. × 1500.



22



23



24

Rewbridge, Dodge and Ayers

Meningitis Due to Endomyces Capsulatus

LIGHT AS A FACTOR IN THE IMPREGNATION OF BRAIN SECTIONS WITH THE AMMONIACAL SILVER SALTS*

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While performing the experiments detailed in a recent paper[1] it was noticed that successful impregnation of the oligodendroglia, microglia and, to a lesser extent, of the neurons appeared to be largely a matter of luck; sometimes they took the silver, sometimes they did not. Already noted and commented upon by other observers, this capricious behavior on the part of these cells has not yet been satisfactorily explained. Good results were generally obtained if the sections were bromurated and impregnated immediately after they were cut; if, however, they were left in their phials in Cajal's formalin bromid for a week or more their oligodendroglia and microglia became increasingly refractory to impregnation and were soon quite undemonstrable.

There might be a number of reasons for this: the formalin might have become acid, the stronger bromid content of the modified Cajal's fixative (6 per cent instead of 3 per cent NH_4Br) used in my experiments might have been to blame, or something might have been dissolved out of these cells by the storage fluid. All these factors could, however, be ruled out: the formalin was kept neutral by the occasional addition of a few drops of 1 per cent Na_2CO_2, the original Cajal's fixative was used in parallel with the modified fluid as a control, ordinary neutral formalin was tried out as a storing fluid, but the sections remained refractory. That anything had been leached out of them seemed improbable, for the blocks from which they were cut had been in formalin for several months and fresh sections from these always gave beautiful results with the oligodendroglia if impregnated immediately after they were cut.

There remained but one readily conceivable factor to be considered: the action of daylight. It was a fact that the best impregna-

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tions had been made during periods of smoky or rainy weather, when the laboratory was so gloomy as to require artificial light; it was also obvious that the sections that had become refractory had been kept on a table in a window where there was often bright sunlight. In order to decide the question the following simple experiment was performed.

Experimental Work

A batch of frozen sections was cut from a brain known to contain an abundance of oligodendroglia and microglia in a good state of preservation (Brain 1 of the series described in the previous paper [1] and taken at necropsy one hour postmortem from an elderly woman). The sections were then divided into two lots: Set L was stored in formalin bromid in a clear glass bottle and placed directly in front of the window where it would get plenty of light; Set D was stored in the same fluid in a brown glass bottle and shut up in a reasonably light-tight cupboard. The two lots of sections stayed in their respective places for about ten days, during which time there was usually bright diffuse light in the window and, on a few days, brilliant sunlight. One very dark day the L-set was exposed to the focussed beam of an arc light for a few minutes, a beam not powerful enough to heat the fluid in the bottle. At the end of the ten-day period a number of sections were taken from each lot and bromurated by twenty-four hours' immersion in 4 per cent NH_4OH at room temperature followed by eight hours in 10 per cent HBr at 38°C in an incubator. While in ammonia the D-set was kept in the dark cupboard, the L-set in the window; while in HBr, both sets were of necessity kept in the dark, as the incubator had no glass door.

Both lots were then impregnated respectively with silver diammino hydroxid and silver diammino carbonate, the complete technic for the procedure is given in the preceding paper.[1] It was at once evident that the two lots behaved quite differently. Even before impregnation it was thought that the D-set had a yellower tinge and that the ammonia through which it had passed remained clearer than that in which the L-set had been exposed to light and in which there was a certain milky opalescence. After impregnation it was seen that the D-set became more intensely stained in both silver solutions, that it gave off clouds of a blackish precipitate in the buffered formalin developer (whereas the L-set did not) and that the

difference in the two sets treated with the carbonate was more noticeable than that in those treated with the hydroxid.

In the carbonate the D-set became fox-red, with a browner medullary portion; the L-set became seal-brown and more diffusely impregnated, with less distinction between the gray and the white matter. At this stage, the difference was so marked that the two sets might be readily sorted out, the one from the other. In the developer they became more alike in their gross appearance and could no longer be readily distinguished, although the D-set was slightly darker than the L-set. In the gold toning bath the D-set grew more bluish violet while the L-set turned a brownish violet; hypo merely accentuated this slight difference.

The final test came under the microscope, after the sections had been counterstained and mounted. It was seen that the D-sections were somewhat more distinctly impregnated with silver diammino hydroxid than were those of the L-set and the neurons were much more distinctly defined; otherwise there was but little choice between them.

The sections impregnated in silver diammino carbonate, however, were strikingly different; there was apt to be troublesome precipitate in the L-set and the impregnation of the oligodendroglia and microglia was decidedly poor, or altogether lacking. Liesegang [2] would probably explain the precipitate by the production of false "reduction centers" (Silberkeime) by the action of light. Of the twenty-four D-sections impregnated in three separate groups in as many separate solutions of the carbonate (in order to rule out differences in making up the carbonate baths) every section of the set showed good impregnation of the oligodendroglia, while four gave unusually brilliant results; there was not a single failure. Of the fifteen sections in the L-set, on the other hand, impregnated in the same solutions at the same time and in the same manner as the others, seven utterly failed to demonstrate the oligodendroglia, two showed but indistinct shadowy forms of these, four impregnated them very slightly, and only two caught them at all well. In the last instance the processes were poorly impregnated, interrupted or stippled. In other words, to put the most charitable construction on the results, the D-set showed one hundred per cent of "takes" in the case of the oligodendroglia, while the L-set showed sixty per cent partial to complete failures and forty per cent quite worthless.

In order to collect further data on the effect of light upon sections to be impregnated with silver diammino carbonate, the same lots of sections used in the preceding experiment were kept in their respective bottles (one in the light of the window and the other in the dark cupboard) until a month had elapsed. They were then impregnated with silver diammino carbonate in the same manner as those of the ten-day set, eleven slides being prepared from each lot. Under the microscope it was found that the results were quite similar to those just detailed; the light-exposed sections were strikingly inferior to those kept in the dark. Although the oligodendroglia was no longer as precisely impregnated as it was in freshly cut sections or the ten-day D-set, it was readily recognizable in every one from the thirty-one-day D-set; nothing better than the faintest suggestion of cytoplasm and processes could be obtained in the case of the one-month L-set. The results were essentially one hundred per cent fair to good impregnation in the D-set, one hundred per cent poor to worthless in the L-set. Furthermore, there was such an increase in precipitation in the latter as to render it practically useless for this reason alone. It was evident, even when sections were kept in a brown bottle in the dark, that the impregnation of the oligodendroglia became less and less precise as time elapsed between sectioning and impregnating. As before, the astrocytes remained relatively unaffected by long exposure to light, while the nerve cells occupied a position midway between these and the oligodendroglia in respect to their light sensitivity.

While this is, of course, too small a series for decent statistical purposes, the result is so clean-cut that the deduction is sufficiently obvious: light distinctly affects the oligodendroglia. Added proof may be adduced from the result of observations made in many untabulated series of unsuccessful impregnations performed on sections kept in the light, during the carrying out of the experiments described in the paper already quoted. That one or two sections of the L-set should show some impregnation of these cells is readily explained by their having become folded or bunched in the fixative, one overlying the other. Some of them were thus partially shielded from the light, while others received its full force. This assumption is further borne out by the fact that those sections in the L-set that did show impregnation of the oligodendroglia were distinctly "spotty"; there were whole fields with no satisfactorily impregnated cells and then

there would be several where they showed partial or complete impregnation. Seven sections of the fifteen, be it remembered, showed no impregnation of the cytoplasm or processes of these cells, while every one of the twenty-four D-sections showed satisfactory or beautiful impregnation.

The microglia cells seemed to share this idiosyncrasy for light, but the results in their case were less striking, probably because the technic was tempered to the oligodendroglia rather than to the microglia, which requires stronger solutions of the silver salts for satisfactory impregnation.

Speculation as to what happens to the cytoplasm of the oligodendroglia under the influence of light would be rather idle at this stage of the investigation; the fact remains that, through its action, these cells distinctly lose their capacity for reducing silver diammino carbonate to metallic silver. As the sets impregnated with silver diammino hydroxid showed somewhat better impregnation in the case of the D-set as compared with the L-set, we may infer as a general rule that one should store frozen sections of brain in brown, rather than in clear glass bottles, and in the dark rather than exposed to light. Apparently light does not permeate blocks of tissue very deeply, for the material used in this experiment had been kept in formalin in the window, exposed to all the light there was; one need not, therefore, be as careful of opaque blocks of tissue as one should be of translucent sections of brain. Obviously, caution would dictate the dark storage of brain tissue in preference to keeping it in the light. There has been a good deal of discussion as to whether impregnation should be performed in the light or in the dark, but I do not recall ever seeing any mention of the effect of light upon brain sections *before* their treatment with light-sensitive silver salts.

Summary and Conclusions

1. Frozen sections of brain tissue should be shielded from light before and up to the time that they are impregnated with silver salts.

2. Light inhibits the impregnation of the oligodendroglia and microglia with silver diammino carbonate and produces troublesome precipitates in the sections.

3. It does not appear to influence the impregnation of the macroglia materially.

4. It somewhat inhibits the impregnation of the cortical neurons with silver diammino hydroxid, after bromuration. This fact may be made use of by technicians desiring to suppress these cells and their dendrites and to accentuate the microglia by contrast.

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MULTIPLE FOCAL SPLENITIS OF GUINEA PIGS*

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The universal use of the guinea pig in laboratory experiments pertaining to bacteriology makes it significant to recognize and understand the spontaneous diseases of this animal. The predilection of *Myobacterium tuberculosis* and *Bacterium tularense* for the spleen following their experimental injection into the body of guinea pigs makes the recognition of spontaneous infection of this organ imperative if one is to avoid errors in the interpretation of splenic lesions following injections.

During the last year I have observed a multiple focal type of splenitis in guinea pigs which, so far as I have been able to ascertain, has not heretofore been described. The disease was encountered largely among the unused or stock animals which were subjected to necropsy examination as a routine following spontaneous death. In three instances material was secured through the kindness of others who recognized the lesions in animals that had died following the injection of various pathogenic bacteria. Twenty-one cases of the disease were distributed throughout the year 1928 as follows: February, two cases; June, two cases; July, three cases; August, one case; September, two cases; October, nine cases; November, one case; and December, one case. Although only twenty-one cases were actually studied, it is quite likely that other cases occurred, since animals, that had died spontaneously and whose bodies were in an advanced stage of putrefaction were not examined. Data were not obtainable on the large number of guinea pigs from the stock pens which were used experimentally by various workers.

PATHOLOGIC CHANGES

Recognizable symptoms were not observed in any of the cases studied. A large stock of animals is maintained under the observation of the attendant throughout the day, and sick or suspiciously

* Received for publication May 6, 1929.

inactive animals are segregated at once. Most of the material studied was obtained from animals that apparently had died suddenly, or during the night. Dead animals in which this condition has been found had usually been well nourished, and generalized demonstrable effects of a disease process were absent.

Grossly the disease appears to be confined to the spleen which is usually noticeably enlarged and striking in appearance, owing to the presence of the large number of lesions. In the material examined, the lesions were of two general types. In eleven cases the spleen possessed enormous numbers of small grayish white foci, seldom measuring more than 0.1 cm. in diameter and usually much less (Fig. 1). These projected slightly above the surface and were found diffusely distributed throughout the body of the organ. In the remaining ten cases the lesions were more variable in size, being from 0.1 to 0.4 cm. in diameter and of a yellowish color. The surface lesions were less numerous, varying from a few to less than twelve in the respective spleens. In a few instances enormously enlarged spleens were observed without gross focal lesions, although sections examined microscopically revealed pathologic changes similar to those characterizing the spleens possessing gross alterations.

The lesions were essentially those of focal necrosis, and a study of the respective spleens in which the disease was spontaneously present revealed all gradations of the necrobiotic process (Figs. 1, 2 and 3). The majority of the lesions appeared to have had their inception in the splenic nodules, the normal elements of which become obliterated as the disease progresses. The earlier changes seem to be those of coagulation necrosis, while in the later stages the center of the lesions shows a marked tendency to caseate. As necrosis proceeds, polymorphonuclear and mononuclear leukocytes appear at the periphery of the lesion, and these cells eventually replace much of the necrotic substance. In the more advanced lesions the majority of the cells in the zone between the necrotic center and the periphery undergo striking retrogression and become clear, with nuclear elements not discernible. The more chronic lesions become definitely circumscribed by a fibrous connective tissue capsule of variable thickness. In many of the lesions this fibrous structure had so fused as to present a hyaline appearance (Fig. 4).

Smears made from the purulent material of the lesions and sections stained by gram's method revealed moderate numbers of gram

positive streptococci. Many sections were stained with carbol-fuchsin in an attempt to demonstrate the presence of acid-fast bacilli, with negative results.

Experimental Methods

Attempts were made to isolate the etiologic agent and to produce the disease experimentally. Many of the cases were unsuited for this phase of the investigation due to advanced postmortem changes, and, as a consequence, only thirteen of the cases were so utilized. The spleen was removed as aseptically as possible under the circumstances, and a portion was macerated in sterile sodium chloride solution. The resultant emulsion was then strained through several thicknesses of sterile cheesecloth and at once inoculated into apparently normal guinea pigs from the stock supply from which the occasional cases of the disease were appearing. In most cases three animals were used, which were inoculated as follows: one received 1 cc. of the emulsion intraperitoneally, one received 1 cc. of the emulsion subcutaneously, and one received 2 cc. of the emulsion by mouth.

Cultures were not attempted from many of the experimental animals that died, because of advanced putrefactive changes. Of the thirty-three guinea pigs inoculated, twenty-five died at intervals of two to one hundred ninety-two days subsequent to inoculation, and eight were killed at the termination of the experiment. The eight that were killed had been inoculated at intervals of ninety-three to two hundred nine days previously. From the data obtained from the inoculation experiments several facts seem apparent, the most important of which are:

1. In seven instances splenic lesions simulating those of the spontaneous lesions were found grossly or microscopically.

2. The microörganism most frequently encountered in the animals that died subsequent to inoculation was a gram positive streptococcus. Many of the strains were hemolytic in character.

3. Purulent peritonitis was often induced by the intraperitoneal and subcutaneous injection of the emulsified inoculum.

4. In only one instance in which the material was introduced orally were there abdominal lesions. This animal, which died after one hundred nine days, revealed multiple abscesses in the walls

of the intestinal tract, and gram positive streptococci were obtained from the lesions. Although the spleen was slightly enlarged, gross lesions were absent; microscopically typical chronic lesions of focal splenitis were present.

5. Much variation seemed to exist in the pathogenicity of the infective material and in the susceptibility of the inoculated animals.

6. The evidence suggests a non-specific gram positive pyogenic streptococcus as a possible etiologic agent in this condition.

7. The exact portal of entry of the organisms responsible for the disease is obscure.

Thinking that there might be some correlation in the occurrence of the spontaneous focal splenitis described and the not uncommon disease of guinea pigs known as "epizoötic lymphadenitis," "lumps," "epidemic lymphadenitis" and "purulent adenitis" described by Boxmeyer,[1] Holman,[2] Hardenbergh,[3] Meyer,[4] Megrail and Hoyt,[5] necropsy was performed on sixty-five animals affected with purulent adenitis. The lot consisted of thirty males and thirty-five females. In eighteen cases the spleen was enlarged, but in none were there gross lesions comparable with those encountered in the spontaneous cases of focal splenitis. In five of the eighteen enlarged spleens, microscopic alterations typical of the chronic type of focal splenitis were observed. The incidence of focal splenitis was highest in October, 1928. In December, the necropsies were performed in which enlarged spleens were found in the eighteen animals. The possibility that the focal splenitis and the epizoötic lymphadenitis existed simultaneously and independently is worthy of consideration. In support of the hypothesis that the diseases are separate entities are the necropsy data in thirty-one of the guinea pigs affected with purulent adenitis which were examined in April, 1929. In none of these was the spleen enlarged. This seems significant in view of the fact that cases of multiple focal splenitis had not been observed since the latter part of December, 1928.

Comment

According to Meyer, the spleen in the disease of guinea pigs designated as pseudotuberculosis, due to a gram negative bipolar rod, is studded with whitish nodules. However, in pseudotuberculosis "the enlarged mesenteric, iliac, periaortic and occasionally inguinal

lymph nodes are transformed into a mass of thick yellowish pus and fibrous tissue." Abscesses are also present in the liver and may involve the kidneys and uterus. In the twenty-one animals from which material was obtained for this study the liver was not affected. The gross lesions in fact were confined entirely to the spleen. The pathologic anatomy and the bacteriologic data seem sufficient to designate the two conditions as separate diseases.

One must be cautious in interpreting the microscopic lesions in the spleen of the seven inoculated animals since it must be borne in mind that the animals used for the inoculations were from the same stock in which the spontaneous cases were appearing. For this reason the assumption that the splenic lesions which were found in the experimental animals had their etiologic inception from the inoculated material, does not seem warranted. Until lesions of the disease can be induced with some regularity by the adherence to a given procedure, it would seem presumptuous to claim that the specific lesions have been produced experimentally.

As further material is encountered it is proposed to make a detailed bacteriologic report of the microörganisms isolated.

Summary

Twenty-one cases of a spontaneous disease of guinea pigs which is characterized by a multiple focal type of splenitis, were studied. Morphologically the disease seems to be confined to the spleen and microscopically the lesions are those of focal necrosis. In an attempt to produce the condition experimentally, thirty-three guinea pigs were inoculated with emulsions of the lesions from thirteen of the cases. Twenty-five of the experimental animals died; the most frequent lesions were those of purulent or fibrinous peritonitis. From many of the inoculated animals which died, a gram positive streptococcus was obtained. The spleens of a few of the experimental animals possessed microscopic lesions similar to those observed spontaneously. The impossibility of ruling out spontaneous cases of the disease in the experimental animals makes it unwise to assume that the specific splenic lesions have been produced experimentally. For the same reason there does not seem to be any correlation between the condition known as purulent adenitis and the splenic disease described.

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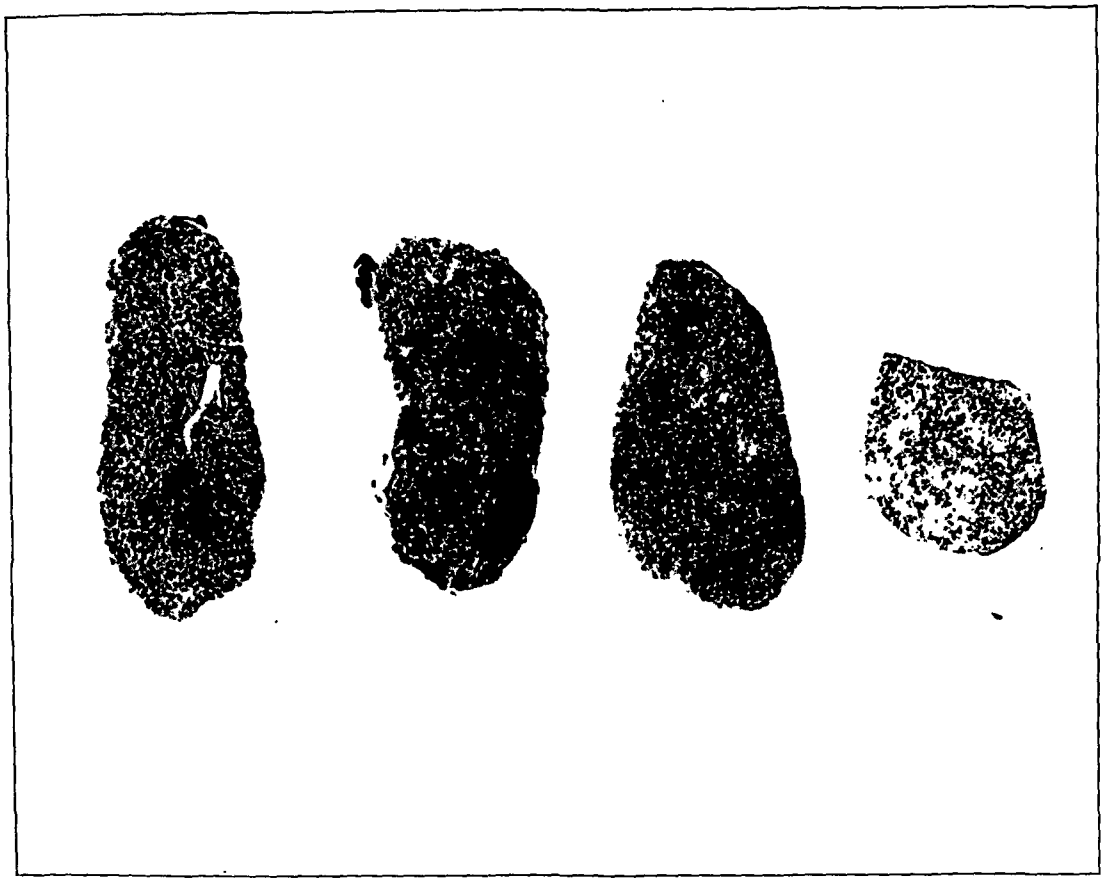
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DESCRIPTION OF PLATES

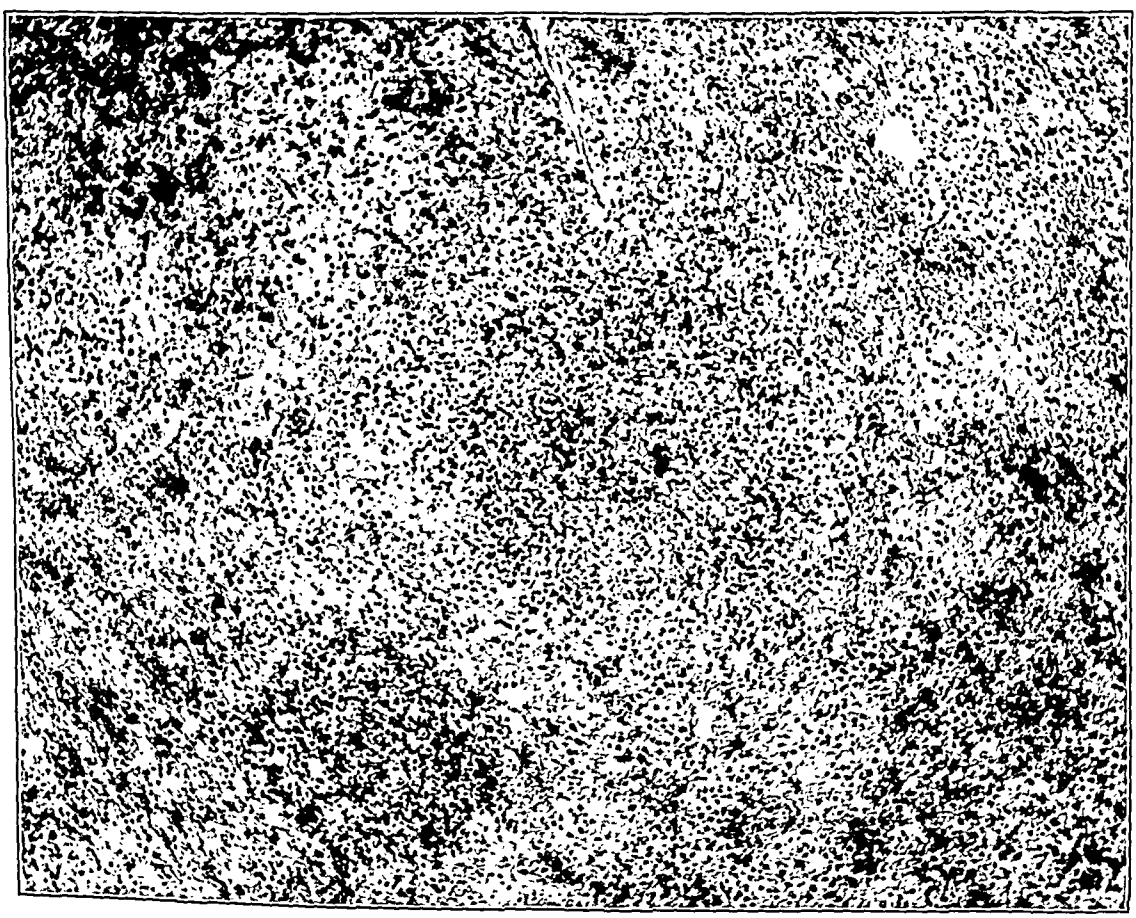
PLATE 74

FIG. 1. Spontaneous splenitis. The multiple miliary character of the lesions is apparent.

FIG. 2. Spontaneous splenitis. An early lesion with beginning necrosis. A peripheral fibrous zone has not developed. × 125.



1



2

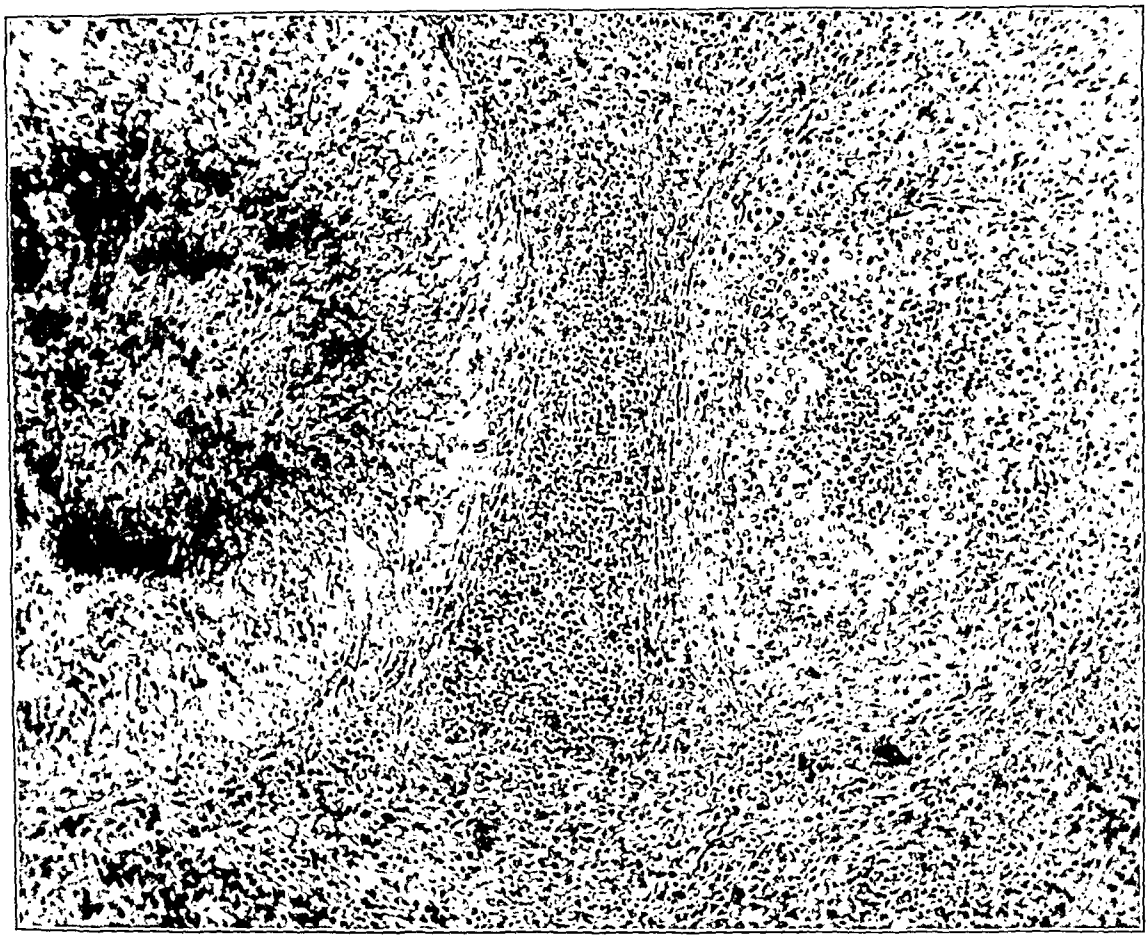
Feldman

Multiple Focal Splenitis of Guinea Pigs

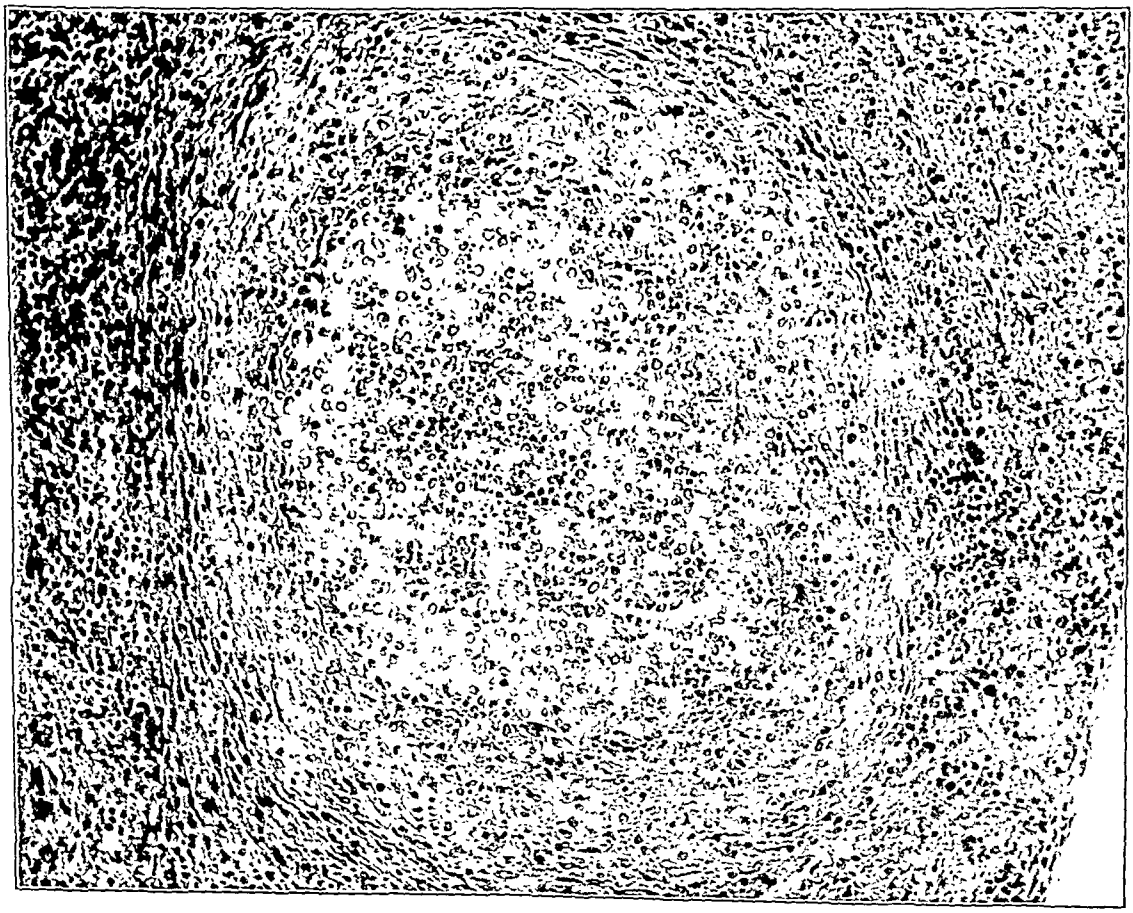
PLATE 75

FIG. 3. Spontaneous splenitis. Multiple lesions of somewhat different character. The one on the left possesses a necrotic center; the cellular integrity of that on the right is still intact. × 150.

FIG. 4. Spontaneous splenitis. The details of a typical lesion are shown. The broad zone of encapsulating cells at the periphery is characteristic. This has a tendency to undergo a hyaline change as the lesion progresses. × 180.



3



4

THE DIAGNOSTIC RELIABILITY OF FROZEN SECTIONS *

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The words "frozen sections" have been heard for many years by some pathologists with a certain feeling of reluctance, distrust, or indifference. Certain pathologists who have not had a large personal experience with all methods of preparation of tissues frequently have approached the subject rather apologetically. Despite this attitude of mind, the method of making frozen sections is at least twenty-three years old, and its reliability has been well tried out here on 208,255 surgical specimens including 28,833 carcinomas.

There are several reasons why some pathologists have failed to realize the value of frozen sections. Such sections are made to best advantage from perfectly fresh, unfixed tissues. Their application to necropsy material or to material which has stood longer than half an hour is not very satisfactory. It has been difficult to obtain good stains; the best is Unna's polychrome methylene blue (Gruebler's) or Terry's recent modification of this stain. Before the World War there was no difficulty in obtaining good stain. During and after the War, stain was made in the laboratories of the clinic; for the last three years Terry's modification of this stain has been used. The stain does not make the diagnosis; it merely saves time in finding the cells. A well trained cytologist could make the diagnosis without stain.

It must be remembered that most pathologists see very little fresh tissue. They are quite naturally and justly wedded to paraffin and celloidin sections. With the material they have had, their technique has been admirable but their studies have been limited largely to the low powers of the microscope. Perfectly fresh unfixed tissues, on the other hand, may and should be studied with oil immersion lenses.

There are many tests of the diagnostic and prognostic reliability both of unfixed and of fixed frozen sections: Let us suppose, for example, that a surgeon removes a lymph node from the region of the

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cecum or sigmoid and the report, based on a fresh, unfixed frozen section, is that the tissue is malignant. If the condition in the resected cecum turns out to be tuberculosis or the condition in the sigmoid, diverticulitis, how long do you think the surgeon would keep his pathologist? Suppose the surgeon removed a lymph node from the neck and the pathologist reported it to be tissue from a case of Hodgkin's disease or of lymphosarcoma and the lymph nodes were excised and found to be tuberculous; again, how long would the pathologist hold his position? If a specimen obtained by biopsy from a bone tumor were reported to be from malignant tissue and the condition were, after amputation, found to be chronic osteomyelitis, or perhaps syphilis, how long would the surgeon continue to have faith in the pathologist's ability? Suppose the surgeon curetted a uterus and the curetted specimen was reported to be from a malignant growth, and on hysterectomy nothing but a polyp or an hypertrophic endometrium was found, how good would the pathologist's reputation be in the minds of his clinical confreres?

It happens occasionally that a pathological diagnosis that is negative for malignancy is followed by the removal of malignant tissue, but this is no fault of the pathologist since he can examine only that tissue which is given to him and it may not contain anything that is malignant.

Frozen sections of fresh, unfixed tissue are so reliable in the hands of a well trained pathologist that he can make the diagnosis of a malignant condition, correctly, from a single cell. This may seem impossible; in fact a well known pathologist with very little experience with fresh tissue told me that it could not be done. The correctness of the statement that it can be and is being done has been proved. Without the pathologist having any knowledge of the specimen or the patient, a colleague, assistant, or technician may place a single cell under the oil immersion lens and may ask the pathologist to make a diagnosis. Let us suppose the cell is in a peripheral sinus of a lymph node, as frequently occurs. Such nodes are often removed so that the surgeon may know whether or not a mass is malignant and just how extensive it is if it is malignant. The report is that the condition is malignant and the surgeon resects the mass; the resected specimen must show the carcinoma if the original report were correct. In my own experience it has never failed. There are two more examples out of many which might be related. Sup-

pose the test specimen is from the uterus and the report is that the tissue is malignant. I have never seen a benign uterus removed after such a report. Suppose it was from a bone tumor and an amputation followed. I have never seen an amputated leg or arm, or any portion of these, that did not show malignancy to be present after such a cellular diagnosis.

There is another test of the accuracy of the method which unfortunately requires years to carry out. The cytologic method of diagnosis has been used for more than eleven years, and hence there has been enough time to carry it out in many cases. Let us suppose that one, or a few, of the malignant cells are found in a case of chronic mastitis or in a chronic gastric ulcer and are recorded as being malignant and the subsequent histories of the patients are followed. What might be expected? Some of the patients would be cured, or at least would be alive for more than eleven years, and we would not know whether the pathologic diagnosis was correct or not; but some of these patients actually return with recurrence of the malignant condition despite radical treatment. In a study of frozen fresh tissue comprising eight hundred sixty-nine freshly resected or excised gastric ulcers, 4.4 per cent contained the "malignant cell" inside of the gastric tubule and 5.2 per cent, inside, or just outside in the immediate stroma. Seven and five-tenths per cent of the first group of patients are dead of unknown causes and 15.2 per cent of the second group are known to be dead of carcinoma within the eleven-year period of study.

There are many other instances of the subsequent or postoperative history revealing the reliability of diagnosis by means of study of fresh, unfixed and fixed, frozen tissue. I bring the subject to the attention of pathologists merely to emphasize three points:

1. Surgeons and other clinicians know the value of frozen sections and have depended heretofore on professors of pathology to furnish candidates for positions in surgical pathology. Such surgeons are going ahead with the use of frozen sections despite the apathy of many general pathologists and are going to develop their own pathologists who may sooner or later become divorced, unfortunately, from the field of general pathology.

2. Pathologists, if interested at all in tissues, are missing a great opportunity to see cells as they are; they are failing to improve their understanding of their own subject.

3. By their lack of clinical interest and failure to utilize fresh tissues they are rapidly becoming thought to be less efficient as microscopic diagnosticians.

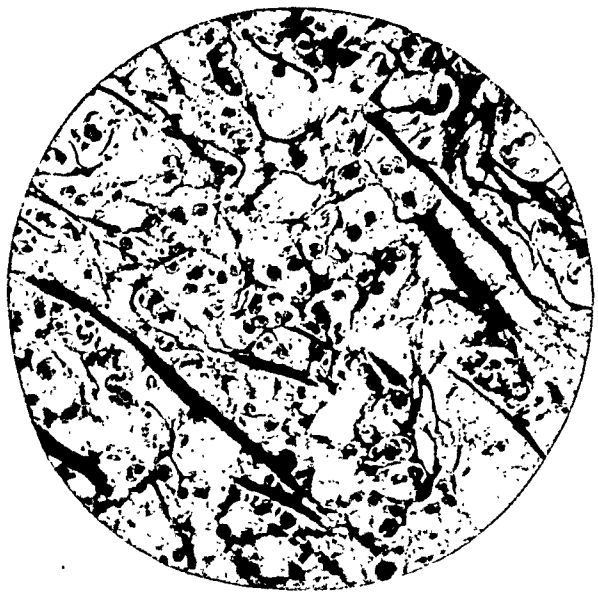
My plea is that pathologists open their eyes to a method which will startle them after a little practice. "I have never seen such beautiful cells" is the usual comment when properly made frozen sections are demonstrated to pathologists who are accustomed to seeing only embedded material.

DESCRIPTION OF PLATES

PLATE 76

Fresh unfixed and formalin-fixed frozen sections.







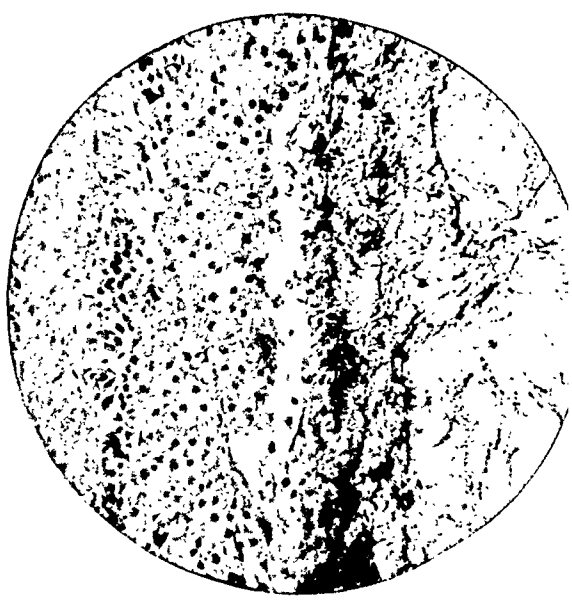


Plate 77

Fresh unfixed and formalin-fixed sections.

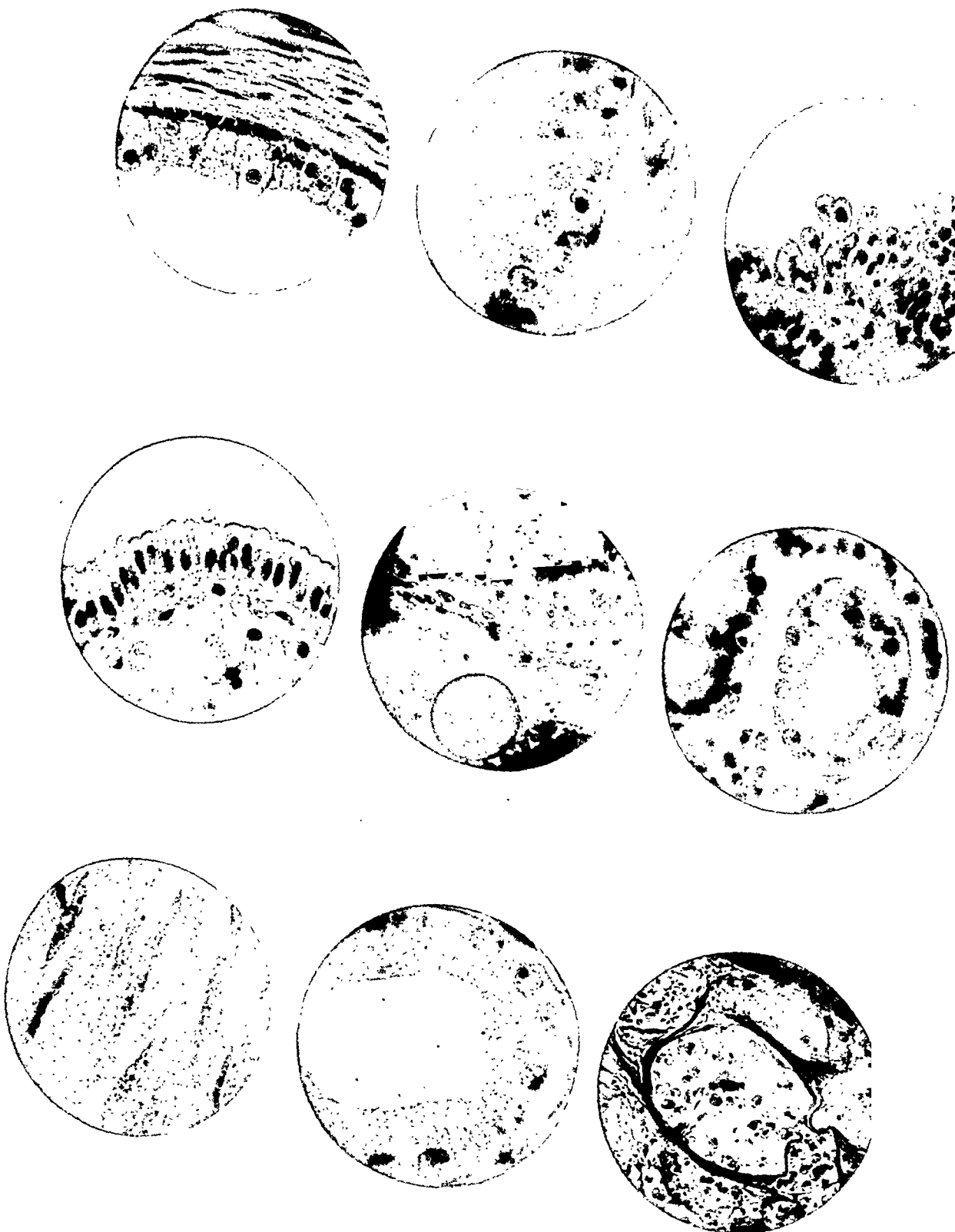
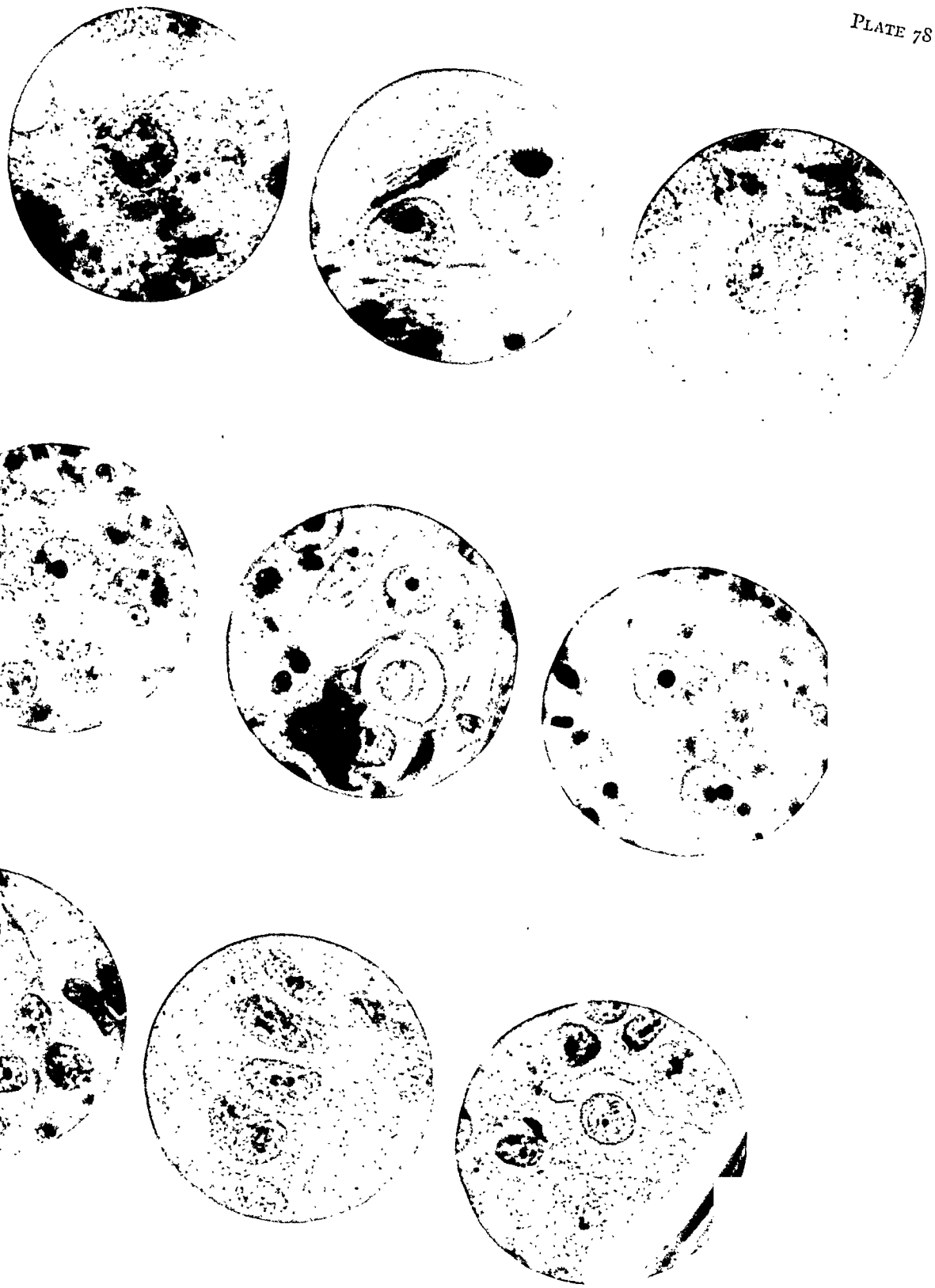


PLATE 78

Malignant cells from fresh unfixed and formalin-fixed frozen sections.



MacCarty

THE PATHOLOGY OF HEMANGIOMA OF BONE *

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The number of cases of hemangioma of bone described in the literature is rather small and the amount of pathological detail remarkably scanty. It is for this reason that we are undertaking here to describe the findings in the seven cases which we have been fortunate enough to study although we are well aware of the limitations of our material. In most of the instances only one slide stained with the usual hematoxylin and eosin is available and in the other cases the amount of tissue removed at operation is very small, usually only a biopsy having been performed. As the clinical histories and roentgen findings have been given completely in a recent article (Bucy and Capp [1]) we shall limit ourselves here to the presentation of the cases in a tabular form (Table I) and the pathological findings will be presented as a general discussion rather than detailed description of the individual case.

Clinically hemangioma of bone is rare. In all we have been able to collect from the literature and our own cases only thirty-nine in which symptoms relative to it were present. Thirteen of these were located in the skull, twelve in the vertebrae and fourteen in other bones of the body, no one of which showed any predominance in frequency of involvement.

On the other hand, Töpfer [2] has recently demonstrated that hemangioma of the vertebrae is a common pathological finding. By careful routine studies in which he sectioned the vertebral column of 2,154 bodies at autopsy, he demonstrated that hemangiomas were present in 257 cases or 11.93 per cent. These varied greatly in size, from a pea up to complete involvement of the entire vertebral body. But in no case was there any history of symptoms referable to the lesion. Doubtless if similar studies of the other bones of the body

* Four of the seven cases included in our study were kindly furnished from the Bone Sarcoma Registry of the American College of Surgeons.

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were made many small hemangiomas would be found lying dormant within the bone.

As will be noted in Table I, as well as in the cases reported by other authors, females are a little more often affected than males, about in the ratio of 55 to 45. There seems to be a definite increase in frequency during the fourth and fifth decades of life although hemangiomas of bone have been found to be congenitally present in the new born (Stamm [3]) and have been found in the bodies of individuals 90 years of age (Makrycostas [4]).

In many cases multiple lesions exist involving either other bones of the body or some of the internal organs. Such cases have been described, especially by Brandt [8] and Stamm. The liver is the organ most frequently involved by multiple hemangiomas. In the case of hemangioma of the vertebra several vertebral bodies are often affected simultaneously, 13.2 per cent of the cases in Töpfer's series.

Hitzrot [9] states that hemangiomas in the long bones usually arise near or at the epiphysis. This is in accord with our observations. However, his statement that hemangiomas arising in the cranial bones usually lie near the suture lines does not seem to be correct. In Case 4 the lesion was a little posterior to the center of the parietal bone.

Grossly the lesion is characterized by a swelling of the bone which is usually of bony hardness although in a few cases the center is soft and on examination of the patient this soft center is found to pulsate (Mauguière [10] and Zajaczkowski [11]). These few cases have occasionally been confused clinically with so-called bone-aneurysm.

If the tissue is incised at operation the hemorrhage is usually so profuse that the surgeon is unable to see the true appearance of the tumor. It can be determined only that there are numerous fine bony trabeculae which are quite friable. If such lesions are examined after removal they will be found to consist of a very vascular tissue lying between the bony trabeculae. Two other types have been mentioned. The tumor removed by Hitzrot had a cystic center which contained a straw-colored mucoid fluid. The wall of this cyst was lined by organizing blood clot beneath which lay the cavernous hemangioma. Such changes in hemangiomas of bone are apparently uncommon, but have been frequently reported in cases of hemangiomas elsewhere, especially in the cerebellum (Cushing and Bailey [12]). The other type is like that of Case 6, which was operated upon by

TABLE I

Case No.	Registered by	Registry No.	Initials	Sex	Age in years	Location	Remarks
1	P. C. B.	940	S. M.	F	62	3, 4, 5, 6 D. vert.	Compression myelitis. Laminectomy. Recovery
2	P. C. B.	941	M. L.	F	49	Rt. scapula	Biopsy. Roentgen therapy. Improvement
3	A. H. Galvin	153	Mrs. J. T. H.	F	38	*Left ilium*	Biopsy. Roentgen therapy. Complete recovery
4	H. Cushing *	230	J. M. C.	F	55	Left parietal	Removal. No recurrence
5	P. E. McNabb	849	C. C.	M	20	Rt. ulna	Removal. No recurrence
6	A. P. Ashhurst †	249	V. D. A.	M	35	Rt. tarsal scaphoid	Curettement. Recovery
7	D. B. Phemister ‡	...	M. McM.	F	40	Rt. clavicle	Removal. No recurrence

* Reported by Dr. Cushing[5] in 1923. † Mentioned by Dr. E. A. Codman.[6] ‡ Reported briefly by Dr. Phemister.[7]

Ashhurst, in which the tissue was not particularly vascular and had the appearance of currant jelly. These cases apparently are very uncommon. We have found no similar cases reported. Hitzrot described a type which he calls "ossifying" and of which the case of Lücke [13] is given as an example. However, ossification seems to be common to all cases of hemangioma of bone.

Of more importance is the fact that although these tumors are frequently found to have broken through the cortex of the bone, the periosteum which has become stretched over the surface of the tumor is unbroken. Cases 5 and 7 were excellent examples of this as will be seen in Fig. 1.

Microscopically the different tumors have an essentially similar structure but certain variations are seen. For the most part the tissue is composed of large cavernous spaces which contain blood cells and are lined with a layer of flat endothelium. The intervascular tissue is loose and rather acellular. The cells are usually spindle-shaped, but some are stellate. The nuclei are ovoid and contain but a small amount of diffusely scattered chromatin. The stroma of connective tissue consists of a loose network of fibers varying greatly in amount. In the tumors reproduced in Fig. 2 it is scanty, while in that shown in Fig. 3*a* it is very abundant. Occasionally large round cells with small pyknotic nuclei and vacuolated cytoplasm are seen. These cells are the so-called "foam cells," and are undoubtedly undergoing fatty degeneration. In the same areas can usually be seen cells whose cytoplasm is occupied by one or two large globules, while in the surrounding tissue, often in the center of a group of these degenerating cells, can be seen globules of fat lying loose among the fibers. In such places there are very few or no cells present. There are often a few small collections of true fat cells. We have never seen a case in which these groups were large or abundant. However, both Makrycostas and Töpfer mention cases in which there is such a large amount of adipose tissue present that the term hemangiolipoma is warranted. Ballance [14] states that in the case he examined only a small amount of lipoid tissue was present.

In Case 1, cells which we interpret as remnants of the destroyed myelogenous tissue are present. These cells have a small, rather compact deeply staining nucleus with a large, rounded cytoplasm which contains granules. By specific methods these granules take the basophilic stain in some cells and the eosinophilic in others. We

believe that these are true myelocytes. We have been unable to find any reference to the finding of such cells in the cases of hemangioma of bone previously reported.

The lining of the vascular spaces as we have previously stated is generally a single layer of flattened endothelial cells, quite comparable to those seen in normal vessels. Such is apparently the rule in these cases (Mauguière, Ballance, and Perman,[15] etc.). However, in one instance (Case 5) the cells are much thicker and the nuclei take a deeper stain. Occasionally places are seen where the lining is two or three cells in thickness instead of one. In this case we have seen no examples of proliferation of the cells out into the lumen such as to occlude it or to form a papillary growth into it. No endothelial hyperplasia beyond that just mentioned has been seen.

The vessels are usually large cavernous spaces which throughout the series of cases are more or less constant in size. In the case reported by Guillain, Decourt, and Bertrand,[16] they were stated to be 3 to 4 mm. in diameter. However, in one instance, a hemangioma of the lower end of the ulna (Case 5), the vessels are much smaller, the lumina corresponding to those of capillaries (Fig. 3*b*). We have been unable to find another example of a capillary hemangioma involving bone. Nauwerck [17] called his case a capillary angioma although it was obviously composed of cavernous spaces. The same is true of the case reported by Ballance.

Microscopically the bony trabeculae within the hemangioma show two changes, degenerative and formative. The degenerative changes are manifest in two ways, both of which seem equally prevalent, necrosis and absorption. In almost any section of a hemangioma of bone can be seen spicules of bone definitely undergoing necrosis (Fig. 4*a*). They are granular, poorly stained and contain no cells or lacunae. Also there are to be seen many spicules with depressions along their surfaces in which can be seen large multinucleated osteoclasts destroying the bone (Fig. 4*b*).

The formative changes are also divided into two groups. The more common of these is evidenced by the many spicules of newly formed bone about whose surface can be seen a closely packed layer of osteoblasts (Fig. 5*a*). The less common is the transformation of fibrocartilage into bone. Strips of fibrocartilage are seen from the edges of which project spicules of newly formed bone. Over the surface of the vertebral arch in Case 1, between the bone and the

muscle is a layer of fibrocartilage. Toward the side of the muscle it gradually passes over into fibrous connective tissue to which the muscle is attached. On the other side the cartilage shows a gradual transition into osteoid tissue and then into bone (Fig. 5*b*). This would seem to explain the means by which the bone extends at the surface if one may generalize from the study of hemangioma of the vertebrae.

Various stages of these changes in the bone itself have been observed by other workers. Mauguière states that in the case of hemangioma of the clavicle which he studied incomplete calcification of osteoid masses was observed. Töpfer, in studying his cases of hemangioma of the vertebrae, saw absorption of bone by osteoclasts as well as the formation of bone by osteoblasts which formed a layer along the bony trabeculae, just as we have observed in our cases. However, no one has noted the generation of bone by the ossification of strands of fibrocartilage unless Mauguière's statement can be so interpreted. Birch-Hirschfeld [18] saw a large amount of cartilaginous tissue in the case he reported but noted no ossification of it. It is not surprising that Töpfer did not observe this process on the surface of the vertebral arch in any of his cases, as the hemangioma was limited to the vertebral body in almost all cases, involved the vertebral arch in only three instances and in none was the hemangioma large enough to produce symptoms. The bony changes in the case of hemangioma of the humerus observed by Ballance are unique. In his case, although the hemangioma was limited to the shaft of the bone, there was marked absorption of the cortex which was demonstrable radiologically as well as very evident in the gross and microscopic preparations. No similar examples are to be found in the literature.

There is nothing in our experience which throws any light upon the etiology of these lesions and as a result we do not feel capable of deciding whether these lesions are neoplasms or vascular malformations. Clinically they are certainly benign tumors and microscopically there is no evidence of malignancy. Mitotic figures are never seen and the stroma is quite acellular, loose and fibrous. Certainly we are dealing with a benign, slowly growing tumor mass that, as a result of its presence and growth, produces a characteristic reaction in the bone in which it arises or which it invades. As we have previously pointed out (Bucy and Capp) there is no reason to

conclude that the changes within the bone itself, both destructive and formative, are a primary part of the neoplastic process, while there is every reason to believe that the changes are only secondary reactive phenomena.

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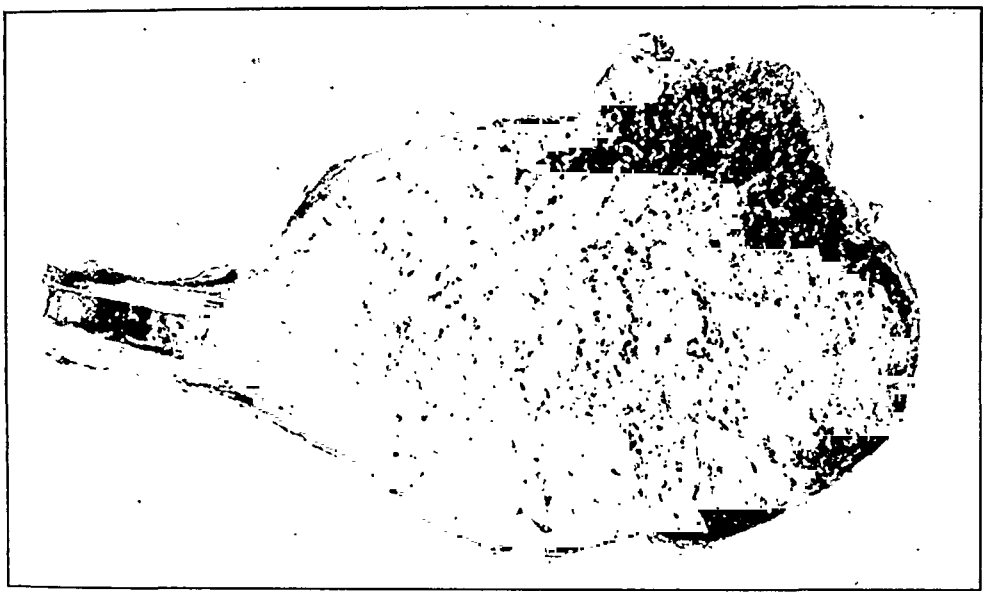
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DESCRIPTION OF PLATES

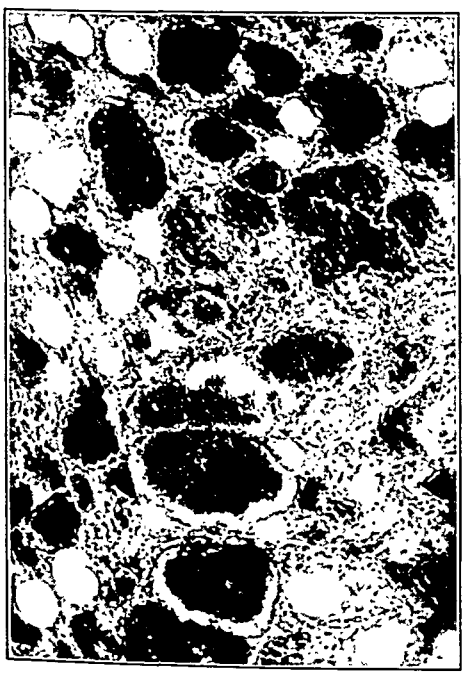
PLATE 79

FIG. 1. Case 7. Hemangioma of clavicle on cut surface. (The saw has broken the cortex in the upper right corner.) Note the complete destruction of the cortex of the bone and the expanded but intact periosteal covering.

FIG. 2. (*a*) Case 2. Hemangioma of scapula. Hematoxylin and eosin. × 70.
(*b*) Case 1. Hemangioma of vertebrae. Note the small amount of intervascular connective tissue, its loose relatively acellular character. The single layer of flat endothelial cells is evident. Hematoxylin and eosin. × 85.



1



a

2



b

PLATE 80

FIG. 3. (*a*) Case 3. Hemangioma of ilium. Large amount of connective tissue between the vascular sinuses. Hematoxylin and eosin. × 85.

(*b*) Case 5. Hemangioma of ulna. Small capillary spaces with thick endothelial lining and a small amount of supporting connective tissue. Hematoxylin and eosin. × 85.

FIG. 4. (*a*) Case 3. A necrotic spicule of bone. × 85.

(*b*) Case 1. A multinucleated giant osteoclast resting in a depression in a bony spicule. × 275.

FIG. 5. (*a*) Case 7. Spicule of bone surrounded by a layer of osteoblasts. Hematoxylin and eosin. × 85.

(*b*) Case 1. Surface of the vertebral arch showing normal bone with underlying normal marrow below and above it a gradual transition through bone, osteoid tissue, fibrocartilage, and finally fibrous connective tissue. The latter is not shown here. × 85.



a

3



b



a

4

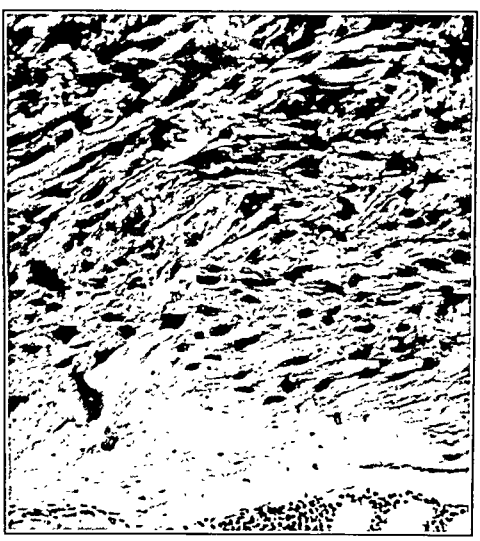


b



a

5



b

CONGENITAL MALFORMATION OF HEART WITH ANOMALOUS INSERTION OF PULMONARY VEINS, ABSENCE OF SPLEEN, SITUS INVERSUS OF ABDOMINAL VISCERA AND OTHER DEVELOPMENTAL ERRORS *

Report of a Case

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It is generally recognized that congenital malformation of the heart is frequently associated with abnormal development of its great vessels and occasionally with anomalies in other organs. In the following case the combination of cardiac malformation and other abnormalities is so unusual that a report of the case seems warranted.

Case Report

Clinical History: Baby Y, male, 7 months old, was suddenly seized with convulsions on Nov. 14, 1925, and died on the way to the Peking Union Medical College Hospital. There was nothing important in his past history except that whenever he cried his lips turned blue.

Necropsy

Gross Examination: This shows a well developed and nourished male Chinese infant, weighing 7,850 gm. The anterior fontanelle is open, 3 by 4 cm. in size, and the posterior fontanelle is closed. Eyes, ears, nose and mouth are normal. No teeth are present. Chest and abdomen are normal. There is marked clubbing of finger and toe nails.

Peritoneal Cavity: No free fluid nor adhesions are present in the peritoneal cavity. The surfaces are smooth and shining. The positions of the abdominal organs correspond to the mirror image of the normal. The liver edge is 5 cm. below the left costal margin and 4 cm. below the right costal margin. The gall bladder is hidden under the liver, its tip just reaching the lower edge of the liver in the

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midline. The round ligament is in the left upper quadrant. The stomach, moderately contracted, is found under the liver in the right upper quadrant. The ascending colon is on the left side of the abdominal cavity and the descending colon on the right, both attached to the mesocolon instead of to the abdominal wall. The appendix, 6 cm. long is shifted to the middle of the lower abdomen. The tail of the pancreas points to the right. No spleen is found. The mesenteric lymph nodes are enlarged and congested. The diaphragm reaches the level of the 5th intercostal space on each side. The inferior vena cava is on the left side of the vertebral column and the abdominal aorta on the right.

Pleural and Pericardial Cavities: Normal.

Heart: The heart is distinctly larger than normal. Viewed anteriorly when the organ lies *in situ* (Fig. 1) the surface is smooth, showing no evidence of interventricular groove. The apex is very broad and rounded. Between the auricular appendages at the base lies the ascending portion of the aortic arch. The pulmonary artery is not seen. On lifting up the apex and throwing the heart upward and backward (Fig. 2) two superior venae cavae are seen, one right and one left, each emptying into its respective auricle. The inferior vena cava opens into the postero-inferior aspect of the right auricle. The pulmonary artery is smaller than usual and has its origin posterior to that of the aorta (Fig. 6). None of the pulmonary veins is found to enter the heart directly. On the posterior surface of the large ventricle there is a small crescent-shaped area surrounded by a zone of fat tissue, suggesting the external surface of a rudimentary right ventricle which, however, is not present. The right auricle is much dilated (Fig. 3). The interauricular septum which forms the left wall of this auricle, is incomplete. It is represented by a small band of tissue bridging the auricle anteroposteriorly and almost horizontally. It is attached to the anterior auricular wall at the auriculoventricular junction and to the posterior auricular wall midway between the openings of the right superior vena cava and the inferior vena cava (Figs. 3, 4 and 5). The right auricle has five openings: (1 and 2) the two interauricular foramina, (3) the opening of the right superior vena cava, (4) the opening of the inferior vena cava, and (5) the auriculoventricular opening. Each of the two interauricular foramina, which are one above the other, is about 1.5 cm. in diameter. The upper one is above the band of the de-

fective interauricular septum and the other below. The opening of the right superior vena cava is on the right side of the upper foramen, looking into both the right and the left auricles. The opening of the inferior vena cava is on the right side of the lower foramen, also looking into both the right and the left auricles. The auriculoventricular opening is guarded by a valve consisting of two cusps not well formed but probably competent. The left auricle is smaller than the right. At its upper border is the opening of the left superior vena cava, and in its posterior wall is that of the coronary sinus (Fig. 5). There is no direct communication between the left auricle and the ventricle. The blood in the left auricle must pass through the interauricular foramina into the right auricle before reaching the ventricle. The ventricle consists of a single chamber narrow anteroposteriorly but wide laterally. There are several large papillary muscles extending from the broad apex upward and backward. This ventricle has three openings: (1) the auriculoventricular opening already described, (2) the opening of the aorta, and (3) the opening of the pulmonary artery. The aorta arises from the left ventricular base and has three normal cusps (Fig. 5). The pulmonary artery originates directly behind the aortic opening. It is much smaller than normal and has a very thin wall like that of a vein. The pulmonary valve is a small funnel-shaped structure with its convex surface on the arterial side. In its middle is the pulmonary opening the size of a pin-point (Fig. 7). The infundibulum is narrow but its musculature is hyperplastic.

Arteries: There are two coronary arteries, the anterior and the posterior. The anterior coronary artery (corresponding to the normal left coronary artery) arises from the aortic wall on the left side, just behind the left aortic cusp (Fig. 5), and passes downward to the anterior surface of the ventricle (Fig. 1). The larger posterior coronary artery (corresponding to the normal right coronary artery) arises from the posterior part of the aortic wall behind the posterior aortic cusp (Figs. 5 and 6). It passes downward for a few millimeters and then gives off three branches (Fig. 6): (1) the left branch, extending to the left along the auriculoventricular groove and downward toward the apex on the left side of the posterior surface, (2) the auricular branch, and (3) the right branch which forms a semicircle around the right half of the auriculoventricular groove. From its proximal portion, it gives off two branches each of which passes

forward to the anterior surface of the heart (Fig. 1). The ductus arteriosus is not found. The pulmonary artery divides into left and right branches which pass directly into the lungs (Fig. 2). From the aortic arch there arise three branches in the order given: (1) the innominate artery which is unusually short, (2) the left common carotid artery and (3) the left subclavian artery (Fig. 1). Between the innominate and the common carotid are two very small twigs, one in front of the other. They are not over ½ mm. in diameter and pass straight upward (Fig. 1). They are probably the thyroidea ima arteries. The descending aorta is found on the right side of the spinal column. The celiac axis, the gastric, splenic and left gastro-epiploic arteries are absent. The superior mesenteric artery arises just a little below the level of the renal arteries (Fig. 9). It passes downward behind the duodenum, where it divides into two branches, the gastrohepatic and the duodenal. The gastric branch runs along the greater curvature of the stomach and sends a few small twigs into the pancreas. The hepatic branch bifurcates shortly before it enters the liver. The left branch, just before entering the liver, sends off a small twig into the wall of the gall bladder. The duodenal artery is much shorter and smaller than the gastrohepatic and supplies only the duodenum. The proximal half of each hypogastric artery is patent.

Veins: There are two transverse pulmonary veins, one above the other (Fig. 2). The upper one which receives blood from all the lobes of both lungs, has no external connection whatever but is connected with the lower transverse vessel through anastomosing vessels in the lung tissue. The lower transverse vessel joins with the large gastric vein along the left side of the lower part of the esophagus and the lesser curvature of the stomach. This gastric vein joins in turn with the portal vein just before the latter enters the liver (Figs. 2 and 10). Thus the blood from the pulmonary vein must pass through the liver before it reaches the heart again. The left descending and ascending azygos veins join together at the level of the fifth thoracic vertebra and empty into the left superior vena cava (Figs. 1 and 2). The right ascending azygos vein passes upward and joins the left azygos before the latter enters the left superior vena cava. The connection between the five or six right upper intercostal veins and the right superior vena cava was unfortunately destroyed at necropsy. The left and the right mammary veins empty into their respective

superior venae cavae. The inferior vena cava is found on the left side of the spinal column but as it passes through the fissure on the left of the Spigelian lobe of the liver it takes a sharp turn toward the right and receives blood from the three hepatic veins (Fig. 10). It then passes through the opening in the diaphragm and enters into the right auricle at its base. The right renal vein crosses the aorta on its anterior surface (Fig. 8). The right spermatic vein empties into the right renal vein.

Lungs: Each lung has three lobes.

Liver: The size of the liver is normal and its shape rectangular (Fig. 12). The left lobe is thick with rounded edges, while the right lobe is thin and can be rolled upon itself. There is no caudate lobe. The tip of the Spigelian lobe points upward and backward. The quadrate lobe is represented by a slight swelling on the lower part of the posterior surface of the left lobe. The fissure for the inferior vena cava is on the left of the Spigelian lobe and the fissure of the ductus venosus is on the right. Below the transverse fissure is the wide, shallow fissure for the gall bladder which is on the right of the swelling of the quadrate lobe. No umbilical fissure is found externally, but when the swelling of the quadrate lobe is cut through, a canal is exposed containing the round ligament.

Gall Bladder and Bile Ducts: They are normal.

Pancreas: The position of the pancreas is reversed, with its head pointing to the left and its tail to the right. The pancreatic duct opens separately into the duodenum at a point about 1.5 cm. above the ampulla of Vater (Fig. 11).

Gastro-Intestinal Tract: The esophagus is normal. Its position is slightly shifted to the right; but its relation to the trachea is not changed. The stomach is transposed to the right. The small and large intestines show very prominent Peyer's patches and lymph follicles.

Adrenal Glands: The left adrenal gland is triangular and is attached to the inferior surface of liver. The right is crescent-shaped.

Diaphragm: The aortic opening in the diaphragm has shifted slightly to the right. The esophageal opening is directly in front of the aortic opening. The opening for the inferior vena cava is on the left of the esophageal opening.

Thymus: The thymus weighs 24 gm. and measures 5.5 by 5.5 by 1.8 cm.

Lymph Nodes: The cervical and other peripheral lymph nodes are normal in appearance. The mesenteric and the retroperitoneal nodes are markedly enlarged.

Other Organs and Tissues: Kidneys, testes, brain, pituitary gland and bone marrow of femur are normal.

Histology: Microscopic examination shows no change of importance, except for the hyperplasia of the lymphoid tissue in thymus, tonsils, and the small and large intestines.

Summary of Findings

Congenital malformation of the heart consisting of one imperfectly separated auricle and one common ventricle; patent septum primum and secundum; transposition of pulmonary artery and aorta; hypoplasia of pulmonary artery; persistent left superior vena cava, non-development of innominate vein; anomalous drainage of the pulmonary veins into the portal circulation; anomalous drainage of the azygos veins into the left superior vena cava; situs inversus of all abdominal organs; absence of celiac axis and anomalous origin of the gastrohepatic and duodenal arteries from the superior mesenteric; patent hypogastric arteries; malformation of the left lung; absence of spleen; hyperplasia of mesenteric and retroperitoneal lymph nodes; persistence of ascending and descending mesocolon; anomalous insertion of the pancreatic duct into the duodenum.

Comment

There are several features in this case on which brief comment may be made. The malformation of the heart is very marked. Although functionally the heart is biloculate, morphologically it is an imperfect triloculate heart. The common auricle is incompletely divided into left and right by a narrow band of tissue representing the septum primum the development of which stopped at the stage when both the ostium primum (interauricular foramen) below and the ostium secundum (foramen ovale) above were present. The septum secundum has never developed. This imperfect formation of the interauricular septum may be secondary to the failure of the pulmonary vein to insert into the auricle. The persistence of the common auriculoventricular orifice is due to the arrested development of the endocardial cushions associated with the non-appearance

of the interventricular septum. The left superior vena cava represents the persistent left common cardinal vein. The normal anastomosis between the left and the right anterior cardinal veins, which later form the left innominate vein, has failed to develop. The pulmonary artery is abnormal both in the degree of its development and in its relation to the aorta. The rudimentary condition of the valve is ordinarily described as pulmonary atresia and is consequent upon the non-appearance of the interventricular septum, as a result of which most of the blood in the common ventricle empties into the aorta. The origin of the pulmonary artery from the posterior aspect of the aorta and its direct course to the lungs can be ascribed to the failure on the part of the endocardial folds of the bulbus cordis to form the usual spiral arrangement. This causes the bulbus to retain its early embryological straight form, resulting in the transposition of the aorta and the pulmonary artery. In this case the transposed vessels are connected with the common ventricle and, in the absence of the interventricular septum, it is difficult to determine whether this transposition might be "corrected" or "uncorrected." The anomalous drainage of the pulmonary veins into the portal system is one of the most interesting findings in this case. In order to understand this unusual phenomenon it is well to refer to the observations of Buell [1] on the development of pulmonary vessels in chick embryos. He finds that in the dorsal endothelial wall of the sinus venosus at the level of the developing lung bud in the chick embryo, there is a proliferation of angioblastic cells extending dorsad until the ventral surface of the gut is encountered. They then grow in all directions over the ventral surface of the gut, contributing to the formation of the splanchnic plexus. This plexus, when fully developed, can be divided roughly into two groups: (1) the group which persists, forms connection with the sinus venosus and later develops into pulmonary veins and (2) the group which forms the cranial and postcaval portions of the splanchnic plexus, and quickly disappears. Occasionally, however, some of the postcaval connections of the splanchnic plexus may persist, while the normal connection between the plexus and the sinus venosus may fail to develop. This results in an absence of connection between the heart and the pulmonary veins, and the formation of a new connection between these veins and the portal system as in the case here described. The absence of spleen has been found in other cases in

association with malformation of the heart and anomalous insertion of the pulmonary veins. Sometimes, instead of the spleen, other organs, such as the kidney, may be absent. The pancreatic opening into the duodenum represents that of the dorsal pancreatic duct (duct of Santorini), which in this case persists. The ventral duct and its opening in the ampulla of Vater are not found, suggesting that probably the ventral pancreas has never developed.

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DESCRIPTION OF PLATES

PLATE 81

FIG. 1. Diagram showing the heart and the relation of its big vessels. The lungs are retracted. Anterior view.

FIG. 2. Diagram showing the vessels of the lungs and the connection of the pulmonary veins with the gastric vein.

KEY TO THE PLATES

A Aorta.
A. B. Auricular branch of posterior coronary artery.
A. C. A. ... Anterior coronary artery.
A. C. A. O. . Opening of anterior coronary artery.
A. V. V. ... Auriculoventricular valve.
Az. V. Azygos vein.
Amp. V..... Ampulla of Vater.
C. D. Cystic duct.
Com. D. ... Common duct.
C. S. Opening of coronary sinus.
Cys. A. Cystic artery.
D. A. Duodenal artery.
Diaph. Diaphragm.
Duod. Duodenum.
Eu. V. Eustachian valve.
F. G. B. ... Fissure for gall bladder.
G. E. A. ... Gastro-epiploic artery.
G. E. V. ... Gastro-epiploic vein.
G. H. A. ... Gastrohepatic artery.
G. V. Gastric vein.
H. A. Hepatic artery.
H. V. Hepatic veins.
I. M. A. ... Inferior mesenteric artery.
I. V. C. Inferior vena cava.
I. V. C. O. . Opening of inferior vena cava into right portion of the common auricle.
In. A. Innominate artery.
L. A. Left auricle.
L. Anas..... Anastomosis between pulmonar veins of the left lung.
L. B..... Left branch of posterior coronar artery.
L. C. C. A. . Left common carotid artery.
L. C. I. A. . Left common iliac artery.
L. Ex. I. A Left external iliac artery.
L. Hy. A.... Left hypogastric artery.
L. Int. I. A . Left internal iliac artery.
L. L. L. Lower lobe of left lung.
L. M. L. ... Middle lobe of left lung.
L. M. V. ... Left mammary vein.
L. P. A. Left pulmonary artery.
L. R. A. ... Left renal artery.
L. R. V. ... Left renal vein.
L. S. A. Left subclavian artery.
L. S. V. C. . Left superior vena cava.
L. U. L. ... Upper lobe of left lung.
Lo. P. V.... Lower pulmonary vein.
Mes. Mesentery.
O. P. D. ... Opening of pancreatic duct int duodenum.
Oesoph. ... Oesophagus.
P. A. Pulmonary artery.
P. C. A. ... Posterior coronary artery.
P. C. A. L. . Lateral branch of the right branch of the posterior coronary artery.

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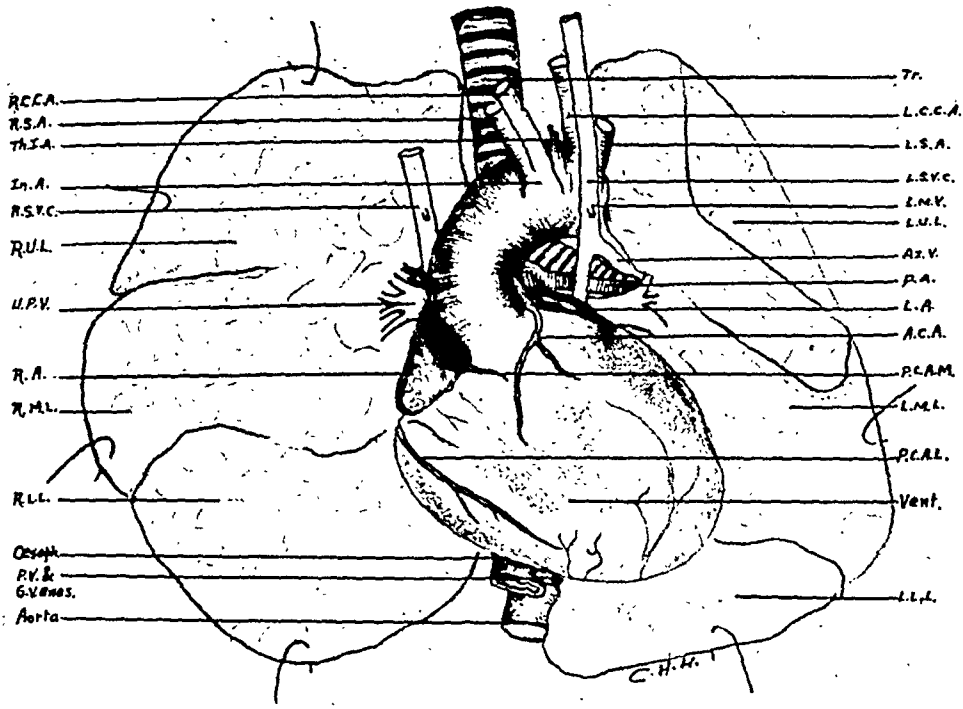

R.C.C.A.
R.S.A.
Th.I.A.
In.A.
R.S.V.C.
R.U.L.
U.P.V.
R.A.
R.M.L.
R.L.L.
Oesoph.
P.V. & G.V. Anas.
Aorta
Tr.
L.C.C.A.
L.S.A.
L.S.V.C.
L.M.V.
L.U.L.
Az.V.
P.A.
L.A.
A.C.A.
P.C.A.M.
L.M.L.
P.C.A.L.
Vent.
L.L.L.
C.H.H.

Fig 1

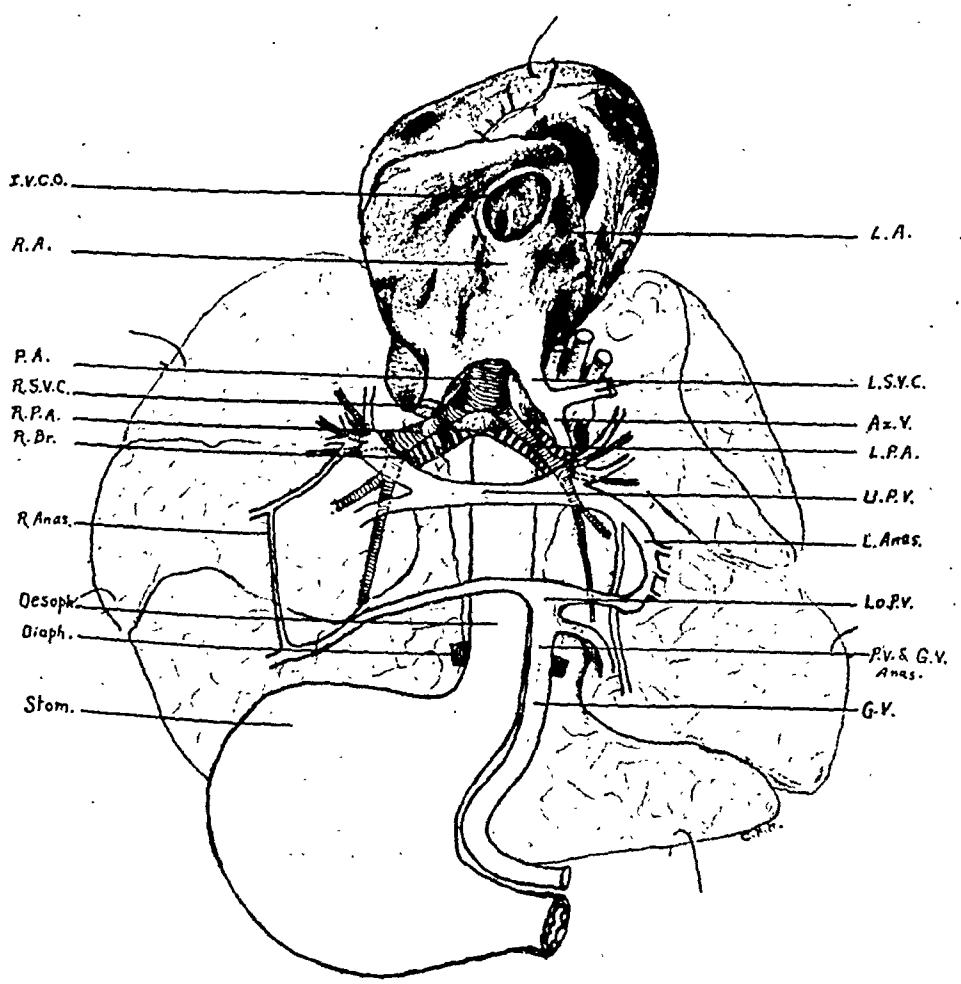

I.V.C.O.
R.A.
P.A.
R.S.V.C.
R.P.A.
R.Br.
R.Anas.
Oesoph.
Diaph.
Stom.
L.A.
L.S.V.C.
Az.V.
L.P.A.
U.P.V.
L.Anas.
Lo.P.V.
P.V. & G.V. Anas.
G.V.
C.H.H.

Fig. 2

PLATE 82

FIG. 3. Posterior and inferior views of the heart with the right auricle cut open. Bristle 1 passes through the auriculoventricular opening and out through a window in the right side of the common ventricle. Bristle 2 passes through the right superior vena cava to the right auricle. Bristles 3 and 4 pass through the left superior vena cava to the left auricle. The lower end of Bristle 3 is seen through the foramen secundum (foramen ovale) and that of 4 is seen through foramen primum (interauricular foramen). Bristle 5 passes through the foramen secundum (foramen ovale) into the left auricle and then through the foramen primum (interauricular foramen) out again to the right auricle.

FIG. 4. Sagittal section of the heart through the right auricle and the common auriculoventricular opening. Bristle 2 passes through the right superior vena cava to the right auricle. Bristle 4 passes through the left superior vena cava to the left auricle. Bristle 6 passes through the opening of inferior vena cava into right auricle and then through the common auriculoventricular opening into the common ventricle. Bristle 7 passes through the aorta into the left portion of the common ventricle.

FIG. 5. Sagittal section of the heart through the left auricle, aortic opening and the left portion of the common ventricle. Bristle 2 passes through right superior vena cava into right auricle. Its lower end seen through the auricular foramen. Bristle 3 passes through left superior vena cava into left auricle. Bristle 8 passes through opening of inferior vena cava into right auricle. Bristle 9 passes through the common auriculoventricular opening.

FIG. 6. Posterior and inferior views of the heart with the auricular appendages and the superior venae cavae retracted in order to show the origin of the pulmonary artery and the origin and distribution of the posterior coronary artery.

KEY TO THE PLATES (*Continued*)

P. C. A. M. Medial branch of the right branch of the posterior coronary artery.
P. V. & G. V. Anas. Anastomosis between pulmonary and gastric veins.
P. Va. Pulmonary valve.
Panc. Pancreas.
Panc. A. ... Pancreatic artery.
Panc. V. ... Pancreatic vein.
Phr. A Phrenic artery
Por V. Portal vein.
R. A. Right auricle.
R. Anas. ... Anastomosis between the pulmonary veins of the right lung.
R. B. Right branch of posterior coronary artery.
R. Br. Right bronchus.
R. C. C. A. . Right common carotid artery.
R. C. I. A. . Right common iliac artery.
R. Ex. I. A. Right external iliac artery.
R. Hy. A. .. Right hypogastric artery.
R. Int. I. A. Right internal iliac artery.
R. L. L. ... Lower lobe of right lung.
R. M. L. ... Middle lobe of right lung.
R. P. A. ... Right pulmonary artery.
R. R. A..... Right renal artery.
R. R. V..... Right renal vein.
R. S. A. ... Right subclavian artery.
R. S. V. C. . Right superior vena cava.
R. Sp. V. .. Right spermatic vein.
R. Sup. A. . Right suprarenal artery.
R. U. L. ... Upper lobe of right lung.
S. M. A..... Superior mesenteric artery.
S. M. V..... Superior mesenteric vein.
Stom. Stomach.
Th. I. A. ... Thyroidea ima arteries (?).
Tr. Trachea.
U. P. V. ... Upper pulmonary vein.
Vent. Common ventricle.

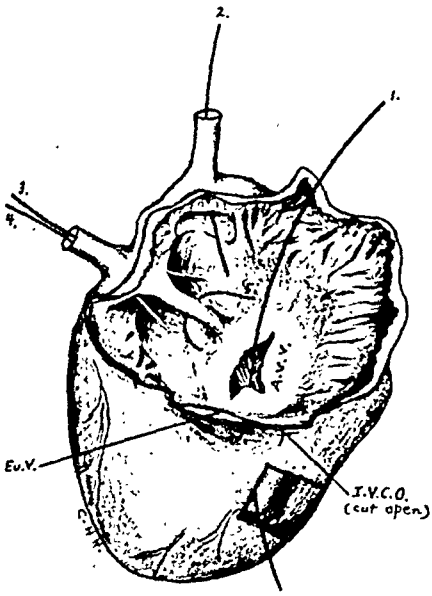


Fig. 3

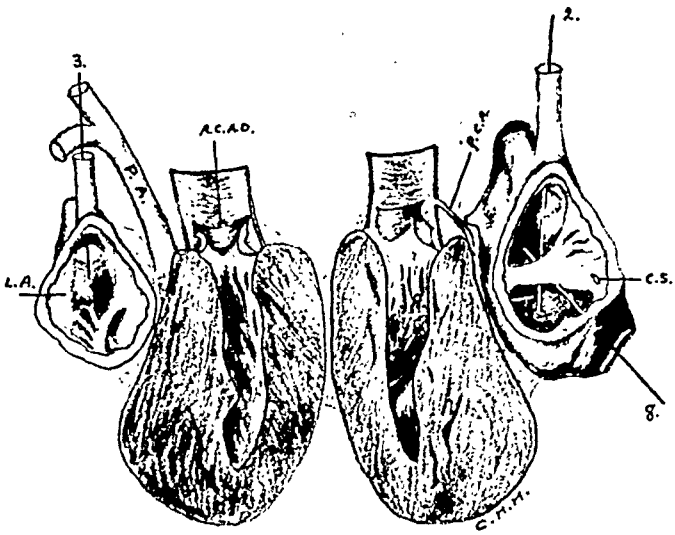


Fig. 5

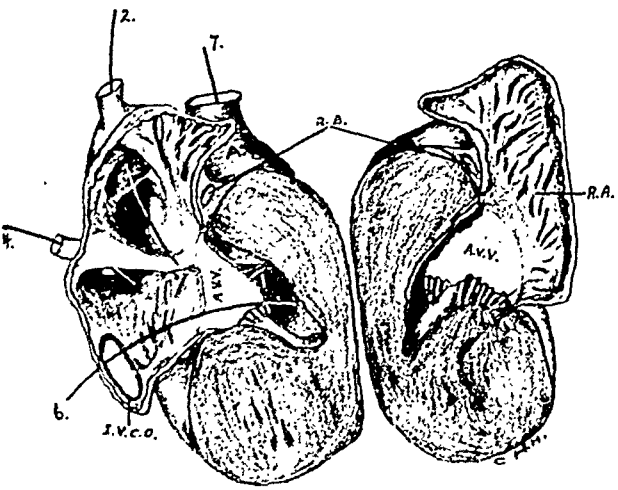


Fig. 4

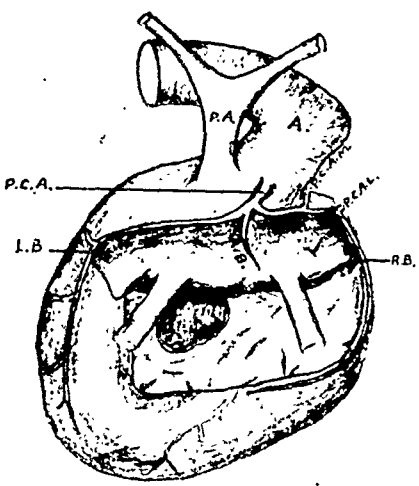


Fig. 6

PLATE 83

FIG. 7. Pulmonary artery and valve, cut open.

FIG. 8. Diagram showing the relations of the renal vessels, anterior view. x (?) Right suprarenal vein. xx (?) left spermatic vein.

FIG. 9. Diagram showing the arteries of stomach, intestine, pancreas, liver and gall bladder.

FIG. 10. Diagram showing the portal vein and its tributaries.

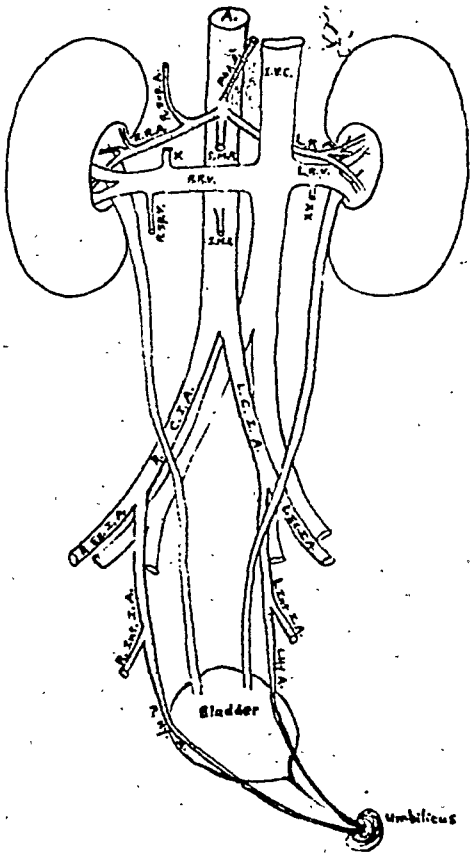


Fig. 8

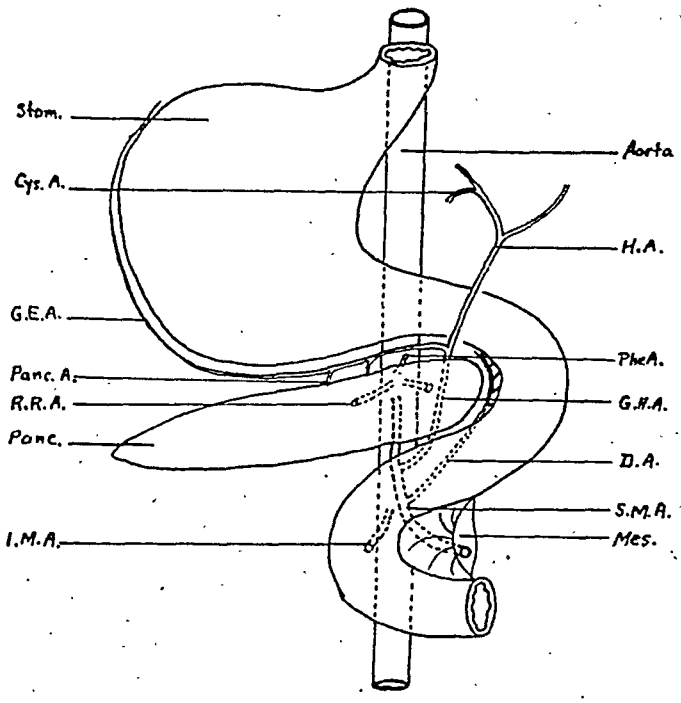


Fig. 9

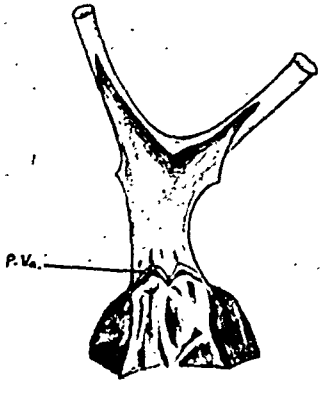


Fig. 7

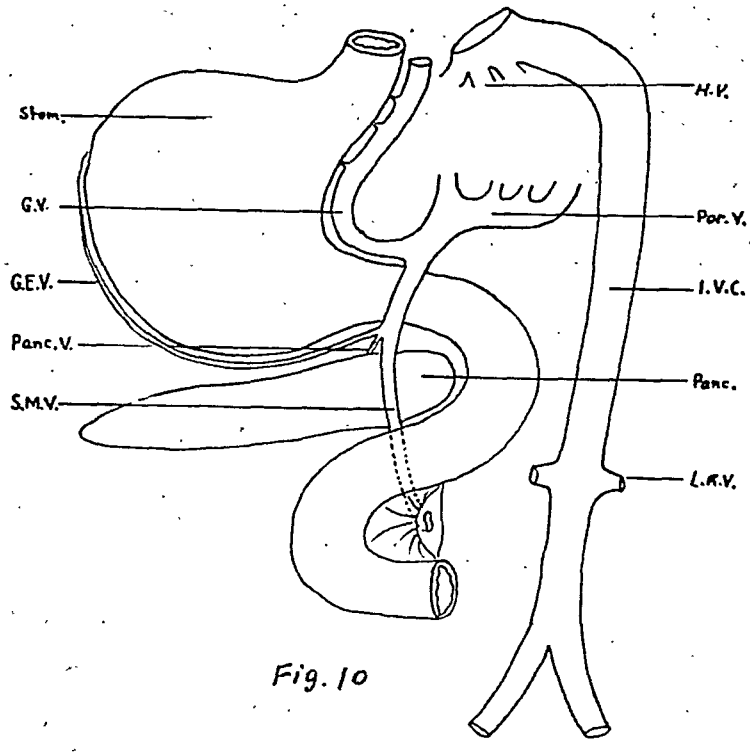


Fig. 10

PLATE 84

FIG. 11. Diagram showing the relation between the openings of the bile and pancreatic ducts.

FIG. 12. Posterio-inferior view of the liver, showing the fissures of the organ. Arrow indicates the direction in which the inferior vena cava passes into the heart.

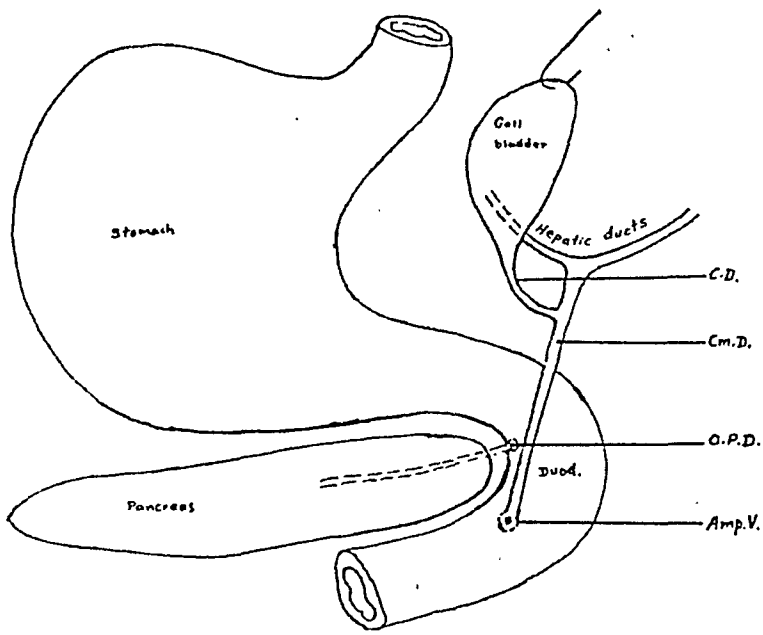

Gall bladder
Stomach
Hepatic ducts
C.D.
Cm.D.
O.P.D.
Duod.
Pancreas
Amp.V.

Fig. 11

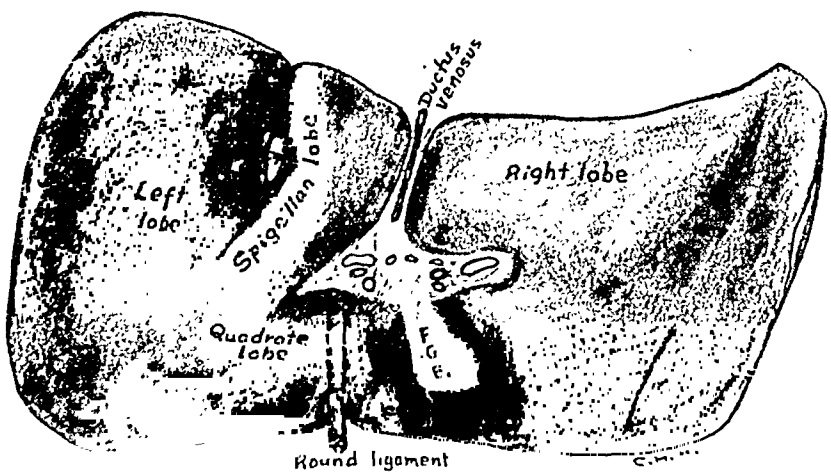

Ductus venosus
Left lobe
Spigellan lobe
Right lobe
Quadrate lobe
G. B.
Round ligament

Fig. 12

INVOLVEMENT OF MEDIUM-SIZED ARTERIES ASSOCIATED WITH SYPHILITIC AORTITIS *

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Much has been written about syphilis of the aorta and about syphilis of smaller arteries, especially arteries of the brain, but syphilis of medium-sized arteries has attracted little attention. In a previous communication [1] the extension of syphilis to the aortic valves was described. The purpose of this paper is to describe the manifestation of the syphilitic process in the other direction, namely in the branches of the aorta. The syphilitic lesions in the aorta seen at autopsy are as a rule old, and hardly any conclusions as to the pathogenesis of the process can be drawn from the old cases. The present study was undertaken as a search for initial or early stages of syphilis in types of vessels whose structures closely resemble that of the aorta. The term medium-sized arteries is used for convenience but it has the sanction of authorities on anatomical nomenclature.

LITERATURE

In 1922 Warthin [2] reported syphilitic lesions in branches of the aorta. His investigation was confined mainly to branches of the descending aorta. He described changes in the carotid in only one case, and changes in the subclavian artery in several cases. In his opinion these changes are essentially the same as are found in syphilitic aortitis. As to the relation of syphilis and arteriosclerosis, he further stated that simple arteriosclerosis of smaller and medium-sized vessels is more common in syphilitics than in non-syphilitics. Warthin, however, did not mention the number of cases examined, nor did he give any details referring to the frequency of the finding of syphilitic changes in the various vessels. MacKenzie [3] stated, (quoting Turnbull) that among two hundred and eighty-eight cases of syphilis, specific lesions were found twice in the coeliac artery and once in the superior mesenteric and splenic artery. Lange,[4] in his

* Received for publication May 17, 1929.

chapter on syphilis of the arteries, stated that the changes in the medium-sized arteries in syphilis are similar to those found in the smaller arteries but described changes of the arteries of this latter type only. Letulle [5] and his co-workers published a case of intermittent claudication which at autopsy showed syphilitic changes of the splenic, common and external iliac, and both tibial arteries. The textbooks of pathology with the exception of MacCallum [6] give detailed descriptions of syphilitic changes of the aorta and smaller arteries, but do not refer to the involvement of the medium-sized arteries.

Material and Methods

Fifty cases which at autopsy showed grossly and histologically syphilitic lesions in the aorta were used for this investigation. Blocks of portions of the innominate, common carotid, superior and inferior mesenteric, common iliac and femoral arteries were taken from these fifty cases and, in the majority of them, from the subclavian also. Owing to the fact that the autopsies were limited to thorax and abdomen, and that syphilitic changes of the vessels in question were more likely to be encountered close to the aorta, the blocks were taken exclusively from these portions. The sections were stained with hematoxylin-eosin, orcein, and a combination of iron hematoxylin and orcein. This combined stain was found especially useful in staining both elastic and nuclear elements in one section. The method of combination of the orcein and iron hematoxylin stain is as follows:

1. Paraffin sections, xylol, alcohol, water.
2. Alcoholic solution of orcein, for one hour in incubator at 37° C. (Orcein, Grübler 0.5 gm., absolute alcohol 40 cc., distilled water 20 cc., hydrochloric acid, C. P., 20 drops.)
3. Water.
4. Acid alcohol, for six seconds. (Alcohol 95 per cent, 200 cc., distilled water 50 cc., hydrochloric acid, C. P., 1 cc.)
5. Water.
6. Iron hematoxylin, 40 minutes. (Solution *a*: Hematoxylin 10 gm., alcohol 95 per cent, 100 cc. Solution *b*: Liquor ferri sesquichlorate 4 cc., distilled water 95 cc., hydrochloric acid, C. P., 1 cc. Equal parts of *a* and *b* are mixed and filtered each time before using.)
7. Acid alcohol. (1 part 2 per cent acid alcohol, 2 parts of water.)
8. Ammonium alcohol, for a few seconds. (30 per cent alcohol with a few drops of ammonium hydroxide 28 per cent.)
9. Water. (Over night.)

10. 95 per cent alcohol to which a few crystals of picric acid are added. 2 to 2½ minutes.
11. Alcohol 95 per cent, absolute, xylol.

Connective tissue and muscle fibers were stained according to Van Gieson's method, and Warthin–Starry's and Levaditi's methods were used in the search for spirochetes.

Results

In a previous paper it has been shown that the earliest manifestation of syphilis of the aorta is in the adventitia in the form of endarteritis obliterans of the vasa vasorum and perivascular infiltration of lymphocytes. These two factors were used as criteria for the presence of early syphilis in medium-sized arteries. Interruption of the elastic lamellae of the media by round cell infiltration or connective tissue in these areas was interpreted as a later manifestation of syphilis.

Innominate Artery: In thirty-three of the fifty cases the innominate artery showed definite signs of syphilis. Some of the vasa vasorum of the adventitia showed a distinct intimal proliferation, resulting sometimes in an almost complete obliteration of the lumina. In some sections there was a varying amount of perivascular infiltration of lymphocytes and plasma cells. In some of the cases a new formation of connective tissue was found in the adventitia. The more pronounced the lymphocytic infiltration, the more connective tissue was observed in the layer. Wherever lymphnodes were found in the adventitia, they seemed hyperplastic. The media in twenty-three cases disclosed an interruption of the elastic lamellae. Some of the vessels presented perivascular infiltration of lymphocytes in the media, but plasma cells were also found in these regions. Older cases showed a new formation of connective tissue throughout the media. This connective tissue was very poor in nuclei and contained small portions of broken up elastic fibers. However, in ten cases the media was free from pathological changes. Some of the sections showed a marked proliferation of the intima with hyalinization and calcification; other sections showed atheromatous cavities, the inner surface of some of which was broken down, leading to atheromatous ulcers covered with thrombi. Some of the sections showed a slight circumscribed proliferation of connective tissue of the subendothelial layer. Throughout this connective tissue a slight infiltration of

lymphocytes was observed. No fatty changes could be made out in these portions.

Carotid Artery: The changes were essentially the same as were encountered in the innominate artery. In twenty-nine cases syphilitic changes were seen in the carotid artery. Whereas in fourteen of these cases the adventitia showed perivascular infiltration of lymphocytes, the media was apparently free from pathological changes. The changes of the intima, especially the circumscribed proliferations of connective tissue, could be studied to a greater advantage in the carotid artery because of the less severe concomitant arteriosclerosis. In serial sections which were cut through the wall of the artery at the site of the circumscribed proliferation of connective tissue of the intima, it was demonstrated that the corresponding portion of the adventitia showed a circumscribed perivascular infiltration of lymphocytes and plasma cells. The adventitia in other portions in some of these vessels was normal. Only the portion of the intima just above these areas offered the circumscribed button-like proliferation of connective tissue. The connective tissue showed a few spindle cells and a slight infiltration of lymphocytes and plasma cells. Between the inner portions of the media and this newly formed connective tissue, a few small-sized blood vessels were seen. There was no multiplication of the lining endothelial cells of these vessels. Degenerative changes were not observed.

Subclavian Artery: The subclavian artery was examined in twenty-nine cases, and in fifteen showed syphilitic lesions. The adventitia alone was the seat of perivascular infiltration of lymphocytes in seven cases. The intima in a majority of cases showed a similar circumscribed proliferation of connective tissue at the site of the perivascular infiltration of lymphocytes in the adventitia. In one case, circumscribed areas were found in the media consisting of endothelial cells, lymphocytes, plasma cells and several giant cells, combined with some foci of necrosis. These areas can be interpreted as gummata. In every other respect the changes were essentially the same as were found in the innominate artery.

Common Iliac Artery: The common iliac artery was involved in ten cases. The changes in the adventitia, media and intima correspond to the findings in the arteries discussed above. In addition to the circumscribed proliferation of connective tissue in the intima, large areas of arteriosclerosis were observed throughout the sections.

Whereas the arteries so far described have an excess of elastic tissue, the arteries which will be discussed subsequently show an excess of the muscular tissue.

Superior Mesenteric Artery: Changes suggestive for syphilis were encountered in the superior mesenteric artery in ten cases. The adventitia showed a varying amount of perivascular infiltration of lymphocytes and plasma cells. A proliferation of the lining endothelial cells of the vasa vasorum was much less often observed than in the arteries discussed above. A few slides showed a rather diffuse infiltration of a few lymphocytes, plasma cells and endothelial cells. The media was much less frequently involved than the adventitia, as changes were found only in one case. Here the changes were present mainly in the outer portions of the media corresponding to the places where most of the elastic fibers were seen, while the inner, more muscular parts showed no changes. The outer part of the media showed an interruption of the elastic lamellae, circumscribed areas of lymphocytic infiltration and some fibrosis. The intima showed in most of these cases a marked, well circumscribed new formation of connective tissue at the site where the perivascular infiltrations of lymphocytes were found in the adventitia. This circumscribed newly formed connective tissue bulged into the lumen, resulting in a narrowing and alteration of the shape of the lumen. There was a splitting of the internal elastic lamellae in several places. Fatty degeneration in the intima or other retrogressive changes were not noted in these circumscribed areas, but were extreme in slides showing diffuse intimal proliferation.

Inferior Mesenteric Artery: Changes characteristic of syphilis were observed in three cases. Here too, mainly perivascular infiltrations of lymphocytes and plasma cells were found in the adventitia, but only very few vasa vasorum showed endarteritis. The media was practically free from pathological lesions, while the intima showed changes similar to those found in the superior mesenteric artery.

Femoral Artery: In only seven cases did the femoral artery show evidence of syphilis, as indicated by a perivascular infiltration of lymphocytes and a few plasma cells. The vasa vasorum showed a moderate degree of proliferation of the lining endothelial cells. There was some fibrosis of the adventitia. The media, in its outer third, showed circumscribed lymphocytic infiltration. In only one

the media in these arteries might easily be accounted for by the rarity of endarteritic changes in the vasa vasorum of the adventitia. If changes were present in the media they were confined to its outer portion, where most of the elastic fibers were present, while the inner portions were free from changes. These findings suggest the propagation of the syphilitic process by way of the vasa vasorum which supply only the corresponding portions of the media.

Throughout the intima diffuse changes characteristic of arteriosclerosis were noted. Fibrosis, hyalinization, calcification, degenerative changes and atheromatous cavities were frequently encountered. In addition to these diffuse changes, circumscribed button-like intimal projections consisting of connective tissue were observed just below the endothelial lining. These lesions were found in the intima at the sites where the adventitia, and in some cases the media, was the seat of a perivascular infiltration of lymphocytes. The diffuse lesions can be interpreted as arteriosclerotic in nature. The severity of these lesions corresponds well with the ages of the patients and substantiates the observations of Hesse,[7] who found severe arteriosclerotic lesions in the arteries of the upper extremities in patients over 40 years of age. The circumscribed, merely proliferative lesions, without degenerative changes, found at the site of syphilitic lesions in the adventitia, however, seem of different etiology. Gurd and Wade [8] believe that syphilis must be recognized as one of the factors leading to proliferation of the intima. Darling and Clark [9] hold that proliferative changes in the intima are a direct response to the syphilitic virus. Whether or not these intimal proliferations really are a manifestation of the syphilitic process cannot be said. Morphologically these lesions show nothing characteristic of syphilis. Stains for spirochetes according to Levaditi and Warthin–Starry did not reveal their presence. There is not enough proof, therefore, to believe that the proliferative changes in the intima are due to syphilis. I am rather inclined to believe that the intimal changes are a non-syphilitic reaction even though brought about by the syphilitic inflammation of the adventitia. The non-syphilitic reaction of the intima probably is a chronic inflammation compensatory to any pathological process in the adventitia, and, in our series, to syphilis.

Conclusions

1. Among fifty cases of syphilitic aortitis, the innominate artery showed syphilitic changes in thirty-three, the carotid in twenty-nine, the superior mesenteric in ten, the inferior mesenteric in three, the common iliac in ten, and the femoral artery in seven cases. The subclavian artery was examined in twenty-nine cases, fifteen of which showed syphilitic lesions.

2. The syphilitic lesions were characterized by endarteritis of the vasa vasorum, and perivascular infiltration of lymphocytes in the adventitia. The media of the elastic type of arteries showed an interruption of the continuity of the elastic fibers and fibrotic areas combined with circumscribed lymphocytic infiltrations. The media of the muscular type of arteries only rarely showed changes.

3. In the intima, circumscribed button-like areas of fibrosis, without degenerative changes, were found very frequently. These are not specific but are chronic inflammatory in nature and might be associated with any type of pathological lesion in the adventitia.

Note: I am deeply indebted to Professor Howard T. Karsner for his valuable suggestions and his aid in taking the photomicrographs, and to Miss Lilian Boker of the Department of Medicine for technical assistance.

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DESCRIPTION OF PLATE

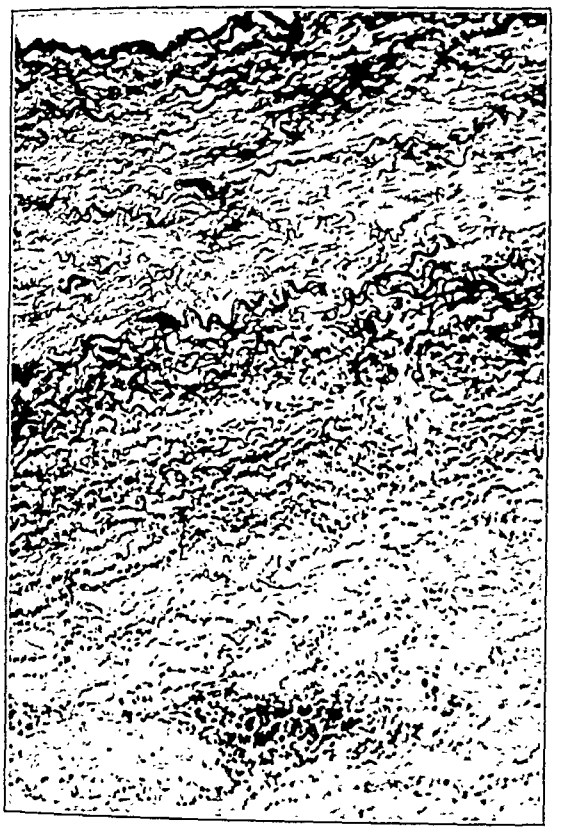
PLATE 85

FIG. 1. Syphilis of the superior mesenteric artery. Note the infiltration of lymphocytes in the adventitia and the inconspicuous interruption of the elastic lamellae in the media. Combination of orcein and iron hematoxylin. × 110.

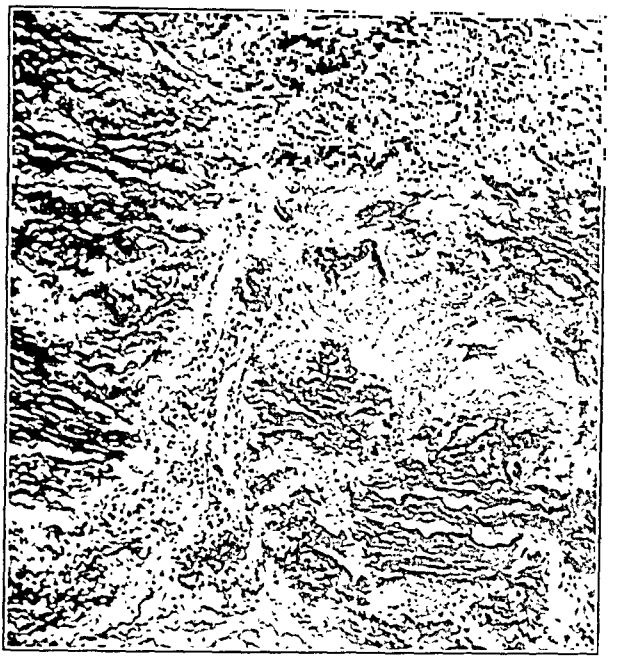
FIG. 2. Syphilis of the common carotid artery. Note the perivascular infiltration of lymphocytes in the media, and the broken elastic lamellae. Combination of orcein and iron hematoxylin. × 110.

FIG. 3. Gumma of the subclavian artery. Combination of orcein and iron hematoxylin. × 120.

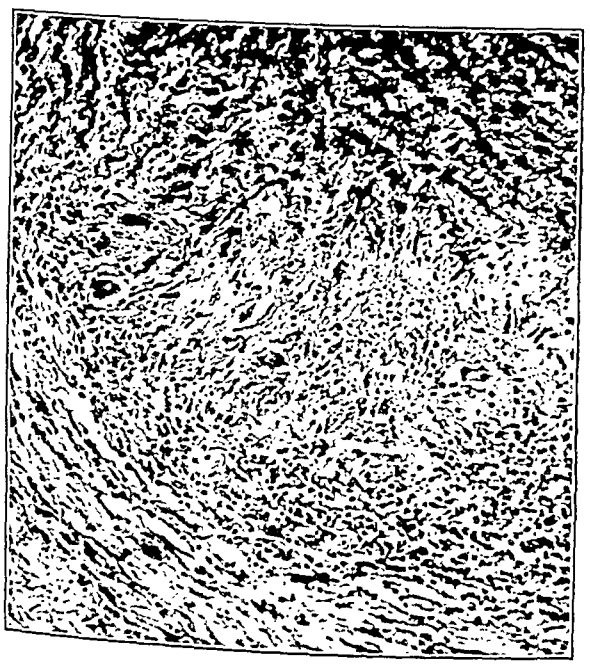
FIG. 4. Syphilis of the superior mesenteric artery. Note the marked intimal reaction. Combination of orcein and iron hematoxylin. × 110.



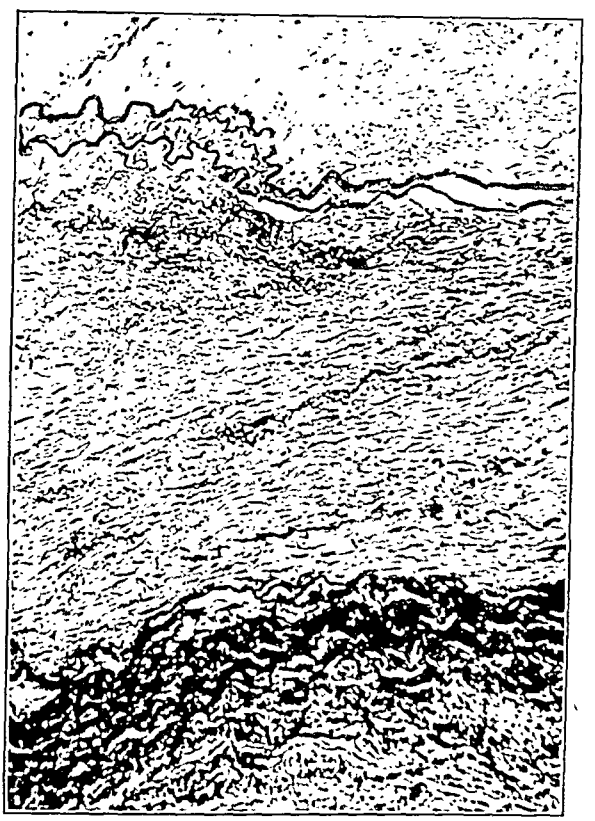
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Saphir

Syphilis of Medium-Sized Arteries

ACCESSORY PANCREASES; REPORT OF A CASE WITH A DISCUSSION OF THEIR PATHOLOGY AND ORIGIN *

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Not less than one hundred and fifty-five instances of accessory pancreas are reported in the literature, but a clear understanding of their origin has not yet been established. The purpose of this paper is to present a case and to discuss the facts and hypotheses concerning the condition.

REPORT OF CASE

A white female, one year old, well developed and well nourished, was admitted to the service of Dr. H. J. Gerstenberger, Babies and Children's Hospital of Cleveland, Ohio, with expiratory and inspiratory laryngeal obstruction. Tracheotomy was ineffective and death occurred twenty hours after admission.

Autopsy three and one-half hours after death revealed an acute fibrinopurulent inflammation of larynx, trachea and larger bronchi, negative for diphtheria bacilli; pulmonary atelectasis, interstitial emphysema of mediastinum; cloudy swelling of the parenchymatous viscera, fatty metamorphosis of liver, accessory pancreas in wall of ileum and recent tracheotomy wound.

Detailed description will be confined to the stomach and small intestine. The stomach is normal except that the mucous membrane is swollen, hyperemic and covered by a thin mucinous exudate. The duodenum is normal. The jejunum and ileum are normal except for slight dilatation by gas, slight hyperplasia of the lymphatic tissue and a discrete firm nodule, 8 mm. in diameter in the wall, 60 cm. below the duodenal jejunal flexure. The serosa overlying the nodule is thick, white and opaque, but not adherent to surrounding organs. The nodule projects into the lumen but the mucosa is normal. In the mucosa near the center is a small depression through which a

* Received for publication May 20, 1929.

probe can be introduced into the substance of the nodule. On cut section, the nodule is of a gray-white color and of a firm fibrotic consistency with islands of softer cellular tissue.

Microscopically the mucous membrane is typically jejunal and normal. The serosa is slightly thickened by dense fibrous tissue. The nodule is composed of typical pancreatic acini, islet cells, intercalated ducts (Fig. 2), larger ducts and fibrous tissue. On the serosal side the fibrous tissue is in excess and infiltrated with a few lymphocytes. The acini in this area are isolated and compressed (Fig. 3). One small lymph node in the edge of the nodule is hyperplastic. Cells cytologically identical with islet cells are irregularly distributed in groups of two to ten. The larger ducts are typically pancreatic. The parenchymatous cells do not exhibit evidence of proliferation. The fibers of the tunica muscularis are separated by a loose fibrous tissue and a few pancreatic acini. Near the exit of the ducts is a small circumscribed mass of tissue (Fig. 4) bounded by smooth muscle and enclosing typical pancreatic ducts and other smaller acini with cells similar to those of Brunner's glands. Stains for zymogen granules were unsuccessful, probably due to improper fixation.

Histologic Features

The general structure of these nodules is such that the diagnosis is evident on microscopic examination. The parenchymatous tissues which may be found are pancreatic acini, islands of Langerhans, larger ducts and glands of Brunner.

The pancreatic acini are usually normal in their structure and arrangement. The acini and ducts do not have the appearance of proliferation and in many cases the acinar cells contain zymogen granules.

The presence of islands of Langerhans is variable. In many reports there is no mention of their presence, in others they were identified with difficulty, while in still others, islands in normal number and size are reported. There is no information concerning the function of such islands as have been observed.

Ducts lined with a typical pancreatic duct epithelium are distributed irregularly throughout the pancreatic acini. In many instances these ducts coalesce into one or two which open into the intestine, or rarely into the bile duct as described by Opie,[1] or main

pancreatic duct as reported by Bean and Dreyer.[2] Nicholson [3] found that these ducts may be directly continuous with ducts lined by cells identical with those of Brunner's glands.

The interstitial supporting tissue may be in the usual amount or may show definite increase with cellular infiltration indicative of chronic inflammation, first pointed out as a prominent feature by Opie. Warthin [4] reported areas of fat necrosis.

In the majority of cases the overlying mucous membrane is typical of that portion of the intestine in which the ectopic mass is found, but in a few the overlying mucosa is typically duodenal, whatever the location.

If the ectopic mass occupies the muscularis, the fibers are often separated by pancreatic or fibrous tissue. This observation is of importance in the consideration of diverticula.

The histologic features of the case here reported do not differ markedly from the above general description. Islet cells are difficult of identification, and fibrosis (Fig. 3) is a prominent feature. The continuity of pancreatic duct epithelium and Brunner's gland epithelium can be demonstrated (Fig. 4).

Theories of Origin

Zenker [5] first proposed that ectopic pancreases have their origin in accessory pancreatic anlagen. Gliński [6] postulated non-fusion of the three primary pancreatic anlagen. Warthin modified these theories with the hypothesis that they represent secondary diverticula snared off from the primary anlagen as they pass through the intestinal wall. Lubarsch [7] believed that inflammatory adhesions may form between the pancreas and adjacent organs with later separation of a small piece of pancreatic tissue. Horgan [8] proposed the same, except that non-inflammatory adhesions are formed during fetal life when all the organs are in close approximation. Taylor [9] assumed that they result from heteroplasia of a portion of the fetal entoderm and places them in the class of "dysontogenetic" structures. Finally, Beutler [11] hypothecated that they are atavistic phenomena.

Thyng [11] has given strong support to Zenker's theory by demonstration of accessory pancreatic anlage in two pig embryos. Horgan has objected to Gliński's non-fusion hypothesis on the grounds that all accessory pancreases would be of large size and located in the

duodenum. This is not valid since if there is a defect in migration there is also very probably an associated defect of development. Further, lengthening and growth of the foregut could carry the anlage distant from the duodenum. A serious objection to Gliński's hypothesis is that it does not explain instances of three accessory pancreases, cases of which are cited by Opie. The reports by Opie of annular pancreases demonstrate that non-fusion may occur.

Simpson [12] supports Warthin's hypothesis on the following points: that accessory pancreases are found most frequently about the papilla of Vater, that they never occur outside the derivatives of the foregut, and that they have been found in the omentum by Warthin, and in the gall bladder by Mann [13] and by von Hedry.[14] As we see it, these observations only point to an origin in the duodenum and do not support Warthin's theory any more than that of Zenker or Gliński.

Cases, such as that of Bean and Dreyer, in which the duct of the accessory pancreas communicates with the main pancreas lend support to Horgan's conception of fetal detachment. The usual absence of anatomical approximation and adhesions makes Lubarsch's theory doubtful except in rare instances.

Taylor has attempted to distinguish accessory pancreases from the adenomyoma of the upper gastro-intestinal tract. He considers the adenomyoma to be a true tumor, characterized by the presence of Brunner's glands with few or no pancreatic acini. Such a distinction has confused his heteroplastic hypothesis for the origin of accessory pancreases. Simpson believes that the adenomyoma is an accessory pancreas in which the acini never developed, regressed or were destroyed by inflammation. Our demonstration of Brunner's glands in an accessory pancreas makes us believe that the cells of the accessory pancreatic anlage are potential acinar, islet, duct or Brunner gland cells.

Beutler's postulation of an atavistic phenomenon is consistent with embryology, but the restriction of all reported accessory pancreases to structures derived from the foregut and its mesentery is a serious objection.

From this review our conclusion must be that in different cases different factors operate: in some, inflammatory or embryonal adhesions with subsequent detachments of a mass of pancreatic cells; in others, development from the primary anlage of Zenker or Gliński,

or the secondary snared-off anlage of Warthin, with the added conception that the cells of these anlage are still undifferentiated entoderm and capable of differentiating into pancreatic acini, islands of Langerhans, ductal epithelium and glands of Brunner. We are unable to accept any theory of heteroplasia, neoplasm or atavistic pnenomena.

Complications and Potentialities

Diverticula: Simpson states that twenty-five of the one hundred and fifty cases of accessory pancreases collected by him were in diverticula. This association is of more than passing interest because of the cause-and-effect relation between the two. From a study of microscopic sections of an accessory pancreas in the intestinal wall, the separation of the muscle bundles leads to the supposition that the wall is weakened at this point and that the diverticulum is secondary to the congenital anomaly. Neumann[15] first proposed such a relationship. Simpson and others support it, placing these cases in the class of true pulsion diverticula. That an accessory pancreas can occur in a true Meckel's diverticulum is undisputed. Koch and Suzuki[16] made a systematic study of fifty cases of Meckel's diverticulum and found accessory pancreatic tissue in two. The finding by Wright[17] of an accessory pancreas in a congenital umbilical fistula, proves such a probable origin.

Since an ectopic pancreas has not been observed in the large intestine, it is unlikely that diverticula here are due to this condition, as suggested by Gibson.[18]

Invagination and Intussusception: When these tumors are located in the submucosa they may occasionally become pedunculated and hang into the intestinal canal as polyps. According to the reports of Hulst[19] and Brunner[20] the intestinal wall was definitely invaginated. Simpson has admirably summed up the whole situation in regard to these processes. He believes that diverticula and invagination are closely related processes, and that which occurs depends entirely on the location of the mass, the amount and character of intestinal peristalsis and the interruption of the muscularis by the mass. Benjamin[21] reports a case of intussusception in which an accessory pancreas was found in the wall of the intussusceptum. Opie and others observed frequent inflammatory changes in these masses, which might well serve as the direct exciting factor of irreg-

ular peristalsis in producing the intussusception. The whole problem appears to be worthy of further study.

Congenital Pyloric Stenosis: Hale[22] reports a small bit of pancreatic tissue embedded in the hypertrophic musculature of a case of congenital pyloric stenosis. Since it had no visible duct, he drew the conclusion that the presence of the ectopic tissue plus its retained secretion led to hypertrophy of the muscle, and suggests that all congenital pyloric stenosis is due to this underlying condition. That a ductless pancreas would secrete and that the pancreatic enzymes would cause only a hypertrophy of muscle is inconsistent with experimental and autopsy observations.

Neoplastic Transformation: Several problems are involved in the relation of these congenital defects to neoplasms. The most important practical consideration is the differential diagnosis at operation. In the case reported by Choisser[23] a small tumor 1.5 cm. by 2 cm. in the wall of the pyloric antrum with enlarged and firm mesenteric lymph node was encountered in a man 57 years of age, complaining of pain in the epigastrium and loss of weight. In instances of this type the differentiation would be extremely difficult. In general an ectopic pancreas is a small round discrete mass in the structures derived from the foregut, and rarely over 1 cm. in diameter.

The relation of ectopic pancreases to adenomyomata has been discussed. Trappe[24] proposed that the carcinoids of the small intestine arise as neoplasms from pancreatic rests, while Saltykow[25] limited their origin to rests composed entirely of islands. The most serious negative objection to this is that carcinoids are most frequent in the appendix, while an accessory pancreas has never been reported in this location. In addition there is the evidence of Masson[26] that the carcinoids of the intestine arise from the chromaffin cells of the crypts of Lieberkuhn.

Finally, there is the question of malignant change in an ectopic pancreas. Branham[27] reports a case in which Welch made such a diagnosis. Nicholson reports another occurring in the stomach and cites two similar cases by Herxheimer[28] and by Seidelin.[29] Although these four cases of carcinoma and one hundred and fifty of ectopic pancreas probably do not represent the total number which have been observed, it would appear that the incidence of carcinoma is higher in ectopic pancreases than in the normal pancreas.

Summary

1. A case of accessory pancreas in the wall of the ileum in a one-year-old child is reported.

2. The mode of origin of these congenital anomalies is either by adhesions of the main pancreatic anlage or from anomalous anlage.

3. Accessory pancreases are in rare instances the underlying cause of diverticula and intussusception and the site of origin of carcinoma.

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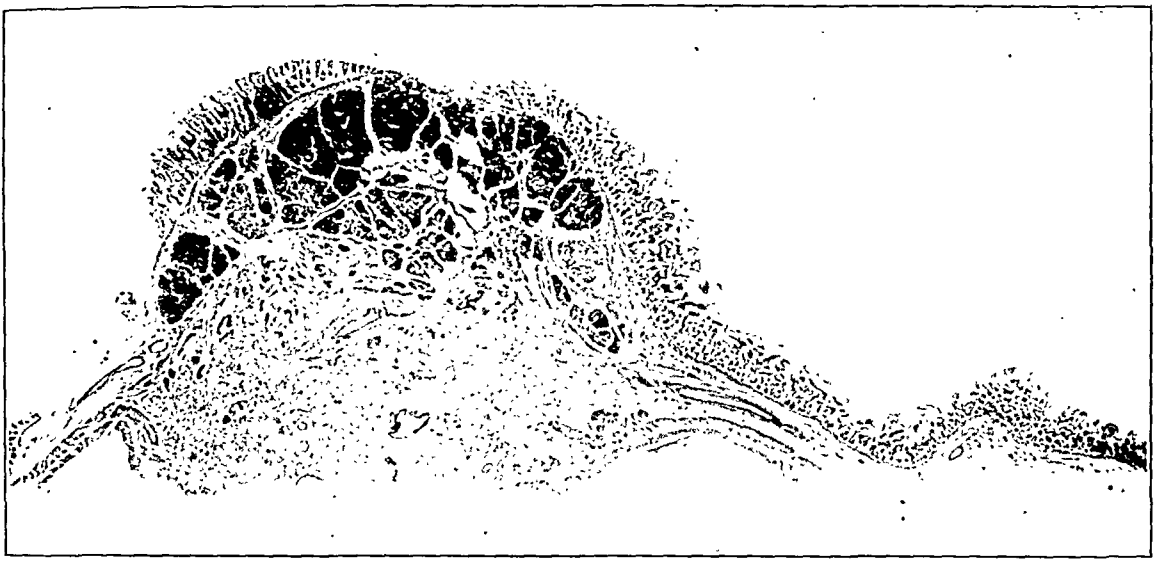
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DESCRIPTION OF PLATES

PLATE 86

FIG. 1. A section through the center of the nodule to show the general location and features. Note the fibrosis and relative absence of acini toward the peritoneum. × 8.5.

FIG. 2. Pancreatic tissue to show pancreatic acini, intercalated ducts and small groups of islet cells. × 100.



1



2

moved from five to eighteen days after operation. In contradistinction to the experiments of Loeb this work is concerned with the early stages of hypertrophy. A quantitative estimation of the mitoses was made in addition to the other usual examination of of the gland.

Experiments

Material and Method: Fifty-one guinea pigs, weighing between 350 and 500 gm., were used. One and one-half lobes of the thyroid gland were removed from each of the animals. In removing the half of the lobe, after the thyroid had been freed from the tissue about it, especially from the artery which sometimes is closely applied to it, the lower pole was clamped and the gland cut through the middle. As a rule there was very little bleeding. The use of a ligature would have interfered with the study of regeneration. The morning after operation the animals were divided into four groups. One of the groups received no treatment, acting as the subjects for the study of normal compensatory hypertrophy and as controls for the other three groups that were fed daily 0.05 gm. of KI, or 5 gr. of anterior pituitary substance (Armour & Co.) or 0.1 gr. thyroid substance (Armour & Co.). The animals were killed by chloroform and their thyroid remnants immediately removed and fixed in Zenker from five to eighteen days after the beginning of treatment. The thyroid remnants were sectioned serially and mounted in rows of eight, nine, or ten. The mitoses in one section in each row were counted and the number thus obtained multiplied by the number of sections in the row. Thus the number of mitoses was obtained for the entire remnant.

Gross Findings: The only observation in the gross specimens of any interest was the greater size and perhaps redder color of the remnants removed from the control and the KI-fed animals as compared to the remnants removed from the anterior pituitary and thyroid-fed guinea pigs.

I. Regeneration

At the site of incision into the thyroid a definite process of regeneration of the acini could be studied. In the earlier glands examined the tissue in this region consisted either of small groups of thyroid cells that had no definite acinar arrangement, or of many very small

acini lined by deeply staining cuboidal epithelium; some of the acini contained tiny droplets of colloid, while others did not. There also were larger irregular acini usually without colloid but containing many polymorphonuclear leucocytes or phagocytic cells. The small acini were formed in three ways: by the reunion of the remaining cells of a partially destroyed acinus; by the differentiation of the small groups of epithelial cells; and by the growth of connective tissue into a larger acinus and the subsequent division of this acinus. The stage that follows this, which is denoted by the presence of large acini, irregular in shape with rather tall epithelium and soft or no colloid, is brought about chiefly by proliferation of the epithelial cells. The greater number of mitoses in the areas of regeneration than in the other parts of the gland supports this view. In the glands removed at five, seven, eight and nine days most of the mitotic activity of the epithelium was found in this region. The return of these acini to the type of acini in the adjacent portions of the thyroid gland so that there was an unnoticeable transition between the two was the final stage.

In the untreated animals regeneration occurred fairly rapidly so that by the tenth and eleventh day the areas of regeneration were becoming imperceptible. The thyroid of the animals fed with KI regenerated about as rapidly as the controls, the eleventh and twelfth days being periods when the return to normal was becoming definite. It should be mentioned that an eight-day control gland and one of the nine-day KI glands had regenerated so rapidly as to resemble the picture that becomes more commonly pronounced on the twelfth day. The glands of the animals fed with anterior pituitary and thyroid never approached in our experiments the amounts of regeneration noted in the controls and KI series, so that even in the latest examined periods, regeneration was not complete in the thyroids of these animals. This retardation of regeneration was more marked in the animals fed with thyroid than in those fed with pituitary.

II. Compensatory Hypertrophy

Mitotic Activity: In Table II the number of mitoses found in the remnants of each gland is tabulated. Marked proliferative activity of the acinar epithelium is indicated in the glands of the untreated animals and in the glands of the KI-fed animals, setting these two

groups definitely apart from the glands of animals fed with pituitary extract and thyroid extract. The average numbers of the mitoses are 1105, 763, 206 and 70, respectively. If we do not include the figures obtained in the thyroid remnants removed at the earliest periods, when the effect of the substances fed may not have become manifest and when most of the proliferative activity is confined to

TABLE 1

Mitotic Activity in Thyroid Remnant

No. of days	Control	KI	Pituitary	Thyroid
5	527*	291*	453*	...
7	121*	...	242*	83*
8	0*	758	298	152
9	1158	96	220	228
9	...	308	210	72
10	1752	1612	138	0
11	1108	995	104	...
11	348	67	36	...
12	4560	816	486	40
13	1368	764	82	46
14	...	...	...	92
15	490	3217	352	0
15	0	880	0	0
16	200	27	54	60
18	2959	96	...	...
18	887	...	...	...
Average of all	1105	763	206	70
Omitting *	1348	803	180	69

the areas of regeneration, our averages of the mitoses are 1348, 803, 180 and 69. The variability in the response of the thyroid gland of the guinea pig to the administration of KI is shown by the wide range of the number of mitoses recorded: 3217 and 27.

The mitotic activity in KI administration is not caused solely by the stimulating effect of KI upon the thyroid gland that has been observed by Gray and Loeb,[8] Rabinovitch,[9] and McCordock,[10] but is also caused by the compensatory hypertrophy. In the KI and control animals the remnants at the time of examination were approximately two-thirds the size of the normal thyroid lobe, and in

the guinea pigs fed anterior pituitary or thyroid substance, the remnants were approximately one-half the size of the normal lobe. The figures obtained in the former may therefore be multiplied by three, and those obtained in the latter by four, in order to compare the number of mitoses in compensatory hypertrophy with the number of mitoses found in the unoperated normal guinea pigs. Thus we obtain the figures 4044, 2409, 720, 276 for the controls, KI, pituitary extract and thyroid-fed animals respectively. The average number of mitoses for these KI compensatory hypertrophy animals as compared to unoperated KI animals is over three times the amount obtained by Gray and Loeb,[8] twice that obtained by McCordock,[10] seven times that obtained by Rabinovitch [9] on the tenth day of his experiments, but it is less than the number obtained by Rabinovitch on animals fed with 0.05 gm. KI daily for fifteen days. In this last series Rabinovitch used only two guinea pigs and the figures may be somewhat high. In pituitary feeding and thyroid feeding, the number of mitoses is only very slightly higher in animals in which a part of the thyroid was extirpated than in normal animals.[8, 10] In the animals that received thyroid and pituitary substance, regeneration may partially account for the increased number of mitoses as compared to normal animals, especially in those fed with thyroid. In the pituitary animals, however, there appears to be a slight definite effect produced through compensatory hypertrophy.

There is another very marked difference between the number of mitoses in the iodized guinea pigs and in those treated with anterior pituitary or thyroid substance. In the latter class the number of mitoses is fairly constantly low, whereas in the animals which received KI, there is very marked variation in the number of mitoses in different cases, similar to the variations found in controls. This indicates that variable factors enter and complicate the degree of hypertrophy observed in control and iodized guinea pigs. These will be discussed later.

Size of the Acini: No exact measurements of the size of the acini were made, but in general we can state that approximately the size of the acini was about the same in the controls and KI-fed animals, and that it was smaller in pituitary and thyroid-fed animals.

Size of the Epithelium and the Character of the Colloid: There exists in general a direct parallelism between the height of the epithelium and the consistency of the colloid, except that the increase in the

size of the epithelium was noted a little earlier than the first signs of the softening of the colloid. In the thyroids of the animals fed with thyroid substance no increase in the size of the epithelium and no softening of the colloid were observed. The epithelium here was low cuboidal and the colloid hard, with occasional retraction; only rarely peripheral vacuolization was noted. In the thyroids of those animals which were fed with anterior pituitary substance the epithelium was a little higher and the colloid a little softer than in the guinea pigs fed with thyroid substance. This applies to periods following approximately the tenth or eleventh day, but this difference was slight. In the animals fed with KI and in the control animals definite signs of compensatory hypertrophy were noted on the eighth and ninth days. At this time the epithelium in both approached in some cases the columnar shape and later became definitely columnar; and the colloid in both showed peripheral vacuolization, became soft and even disappeared. In our series of animals the thyroid remnants of the control guinea pigs showed, on the average, slightly more hypertrophy than the remnants of the guinea pigs fed with KI. However, the thyroid glands of two of the KI-fed guinea pigs were more hypertrophic than those of the control thyroids. Again the variation in both series was very great in different cases. In some animals the hypertrophy was slight or absent while in others it was very marked. We found some gland remnants in which the epithelium was high and the colloid soft, in which there was therefore distinct hypertrophy present, although the number of mitoses was low. Usually an increased number of mitoses was associated with the signs of hypertrophy, but rarely exceptions were found for this rule.

Phagocytosis and Lymphocytosis: We can confirm the observations made previously [8] that the number of phagocytic cells in the colloid is greater in animals that have been fed with KI than in the animals of any of the other series. In the control series the acini contained more phagocytes than in the pituitary series, while the colloid in the glands of animals fed with thyroid was invaded by very few phagocytic cells.

No constant relationship between the presence or amount of lymphocytes and the substance fed to the animals could be detected.

Discussion

The delayed regeneration of the incised thyroid gland caused by the administration of anterior pituitary and thyroid substances which we have described might be expected from the previously observed inhibiting effect of these substances upon the activity of both the normal thyroid and upon the thyroid during the process of compensatory hypertrophy as indicated by structural changes. Thus we now find that these two substances produce not only a histological and probably a functional resting stage, but also by their depressing effect upon the proliferative activity of the acinar epithelium cause a delayed regeneration in the gland. Because of the known stimulating effect of KI upon the mitotic activity of the normal thyroid gland, one would suppose that regeneration would occur earlier in the KI-fed guinea pigs than in the control guinea pigs during the process of compensatory hypertrophy. However, the number of animals examined by us at a period between five and nine days is too small for an accurate determination of this question.

As far as compensatory hypertrophy is concerned there can be no doubt, in these experiments at least, that there are marked differences in regard to the number of mitoses, the height of the epithelium and the consistency of the colloid between the thyroid glands of animals fed with anterior pituitary and thyroid extracts on the one hand, and the thyroid glands of animals fed with KI and the controls on the other hand. The former gave the histological picture of resting glands, the latter gave the histological picture of definite hypertrophy.

Taking the series as a whole, the thyroids of the control guinea pigs underwent slightly more epithelial proliferation and hypertrophy and slightly more colloid softening than the thyroids of those animals fed with KI. It must be remembered, however, that there was variation in the animals in each series, possibly somewhat more in the KI animals. These individual differences in intensity of compensatory hypertrophy in normal as well as in iodized guinea pigs has been emphasized before by Loeb.

Among the variable factors responsible for these individual differences in the degree of compensatory hypertrophy the change in weight of the animals during the progress of the experiment is very important. As Loeb, and Rabinovitch [11] have shown, animals that

gain in weight tend to show a more marked hypertrophy and a greater proliferation of the normal gland than animals that remain stationary or that lose weight during the time of the experiment. In our series ten of the controls gained in weight, four remained stationary and none lost weight, while in the KI series, three of the animals lost weight, while four remained approximately stationary and only six gained in weight. This may explain why in our series the average of compensatory hypertrophy was somewhat greater in the control series.

Furthermore, it is possible that the action of KI upon the thyroid gland does not reach its maximum until after sixteen to eighteen days, which is the period of marked proliferation in normal unoperated guinea pigs as the experiments of Gray and Loeb, and Rabinovitch indicate. Considering the importance of these variable factors the number of animals used by us in the control and KI series is not yet large enough to determine whether the compensatory hypertrophy in the early periods following removal of a great part of the thyroid gland is somewhat greater in the controls than in the iodized animals. On the other hand, we can state that even in these early periods administration of anterior pituitary as well as thyroid substance prevents compensatory hypertrophy, while KI does not have such an inhibitory effect.

Conclusions

1. Regeneration of thyroid tissue after an incision occurs rapidly in control and KI-fed animals.

2. Regeneration is delayed by the feeding of pituitary extract and thyroid extract.

3. Early compensatory hypertrophy is markedly inhibited by pituitary extract and thyroid extract but only slightly by KI.

4. The possible causes of a slight diminution in the average hypertrophy in the KI series which we observed at early periods, are discussed.

5. Compensatory hypertrophy may appear as early as eight days following the removal of parts of the thyroid glands.

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AUTO- AND HOMOIOTRANSPLANTATION OF CROSS-STRIATED MUSCLE TISSUE IN THE RAT*

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The aim of these experiments has been, in the first place, to analyze the difference between autotransplantation and homoiotransplantation of cross-striated muscle and, secondly, to study the changes which the muscle pieces undergo at various periods following transplantation.

The literature on muscle transplantation is extensive, but the large majority of these experiments were carried out from surgical points of view; they concerned mainly autotransplantation. Some authors found that the transplanted muscle tissue became completely necrotic soon after transplantation; others found that it showed peripheral regeneration for some time and then degenerated, and still others concluded that it remained alive *in toto*, especially if nerve connections were soon established between host and transplant. It will not be necessary to review here the literature in detail; instead, we can refer to the review of Eden in Lexer's [1] book on transplantation. For our purposes it will be sufficient to refer specifically to the experiments of Saltykow,[2] who carried out an extensive series of autotransplantations in rats and mice, of Askanazy [3] and Jores–Köln,[4] and of Shinya,[5] the only author who compared carefully the results of auto- and homoiotransplantation of striated muscle tissue.

Concerning the differences between auto- and homoiotransplantation in general, Loeb [6] and his associates studied auto-, syngenesio- and heterotransplants of various organs. In a summary of his own investigations and of those of his collaborators, he concludes that the reactions against the transplant are quantitatively parallel to the relationship between host and donor and that the behavior of the lymphocytes especially, but also of connective tissue, can be

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used as a quantitative indicator of that relationship. The autotransplants have the greatest degree of efficiency in preserving their integrity, in maintaining inviolate the relationship between parenchyma and stroma and in preventing the invasion of the transplant by lymphocytes and connective tissue of the host. This efficiency decreases as we pass from syngenesio- to homoiotransplants. The lymphocytic reaction is lacking in the autotransplant and it increases progressively as the relationship between host and transplant becomes more distant, reaching a maximum in the homoiotransplant. It decreases in the heterotransplant if the toxic action of the body fluids has caused a marked injury of the transplanted tissue; otherwise the reaction is very pronounced in these cases and, in addition to lymphocytes, polymorphonuclear leucocytes may be attracted.

Saltykow made free autotransplantations of muscle and observed that, at first, the muscle degenerated. At five days he found evidence of far advanced regeneration, the muscle nuclei being now more numerous and larger in size. The contractile substance surrounding the nuclei degenerated into small, granular masses and disappeared. Gradually there occurred a formation of spindles and from these, narrow muscle fibers were produced. He also observed that the fibers closest to the periphery were either best preserved or showed the greatest regeneration, owing to their greater proximity to the blood vessels of the surrounding tissue. At two weeks the fibers were wider and the nuclei, although still increased in number, were situated in the periphery of the fibers and had become less numerous. A few fibers showed suggestions of cross-striations. At four weeks, the new fibers differed from the original ones only by being a little narrower. During the later stages a simple atrophy took place. At the beginning of the fourth month scarcely any cross-striated muscle fibers remained. He explains the final atrophy as being due to lack of use of the transplanted tissue.

Askanazy transplanted muscle into the brain and observed regeneration at the end of the first week, but at the beginning of the third week he found signs of atrophy in most of the newly formed fibers. At two months, only the last remnants of the transplanted muscle fibers showing a fibrillar structure were seen. According to Jores, faradization of the autotransplanted muscle piece increases the regeneration of the peripheral muscle fibers and the temporary preservation of parts of the transplants.

Shinya was the only author who, besides studying the fate of the muscle transplants, also compared the histological pictures resulting from auto- and homoiotransplantation of cross-striated muscle into peripheral nerves. He concluded that muscle can be transplanted to the same host as well as to unrelated hosts of the same species with good results. In accordance with the previous findings of Loeb and his collaborators, Myer[7] and Sale,[8] in the case of other organs, Shinya observed that the lymphocytic reaction is much stronger in the homoio- than in the autotransplant, and also that the muscle fibers maintain themselves much longer in the latter than in the former. Furthermore, the intensity of new formation of muscle fibers was much less and the newly formed fibers degenerated much earlier in the homoio- than in the autotransplant. After thirty-five days he found that the autotransplanted muscle had disappeared completely and had been replaced by connective tissue. In the homoiotransplants, at twenty-seven days there was merely a necrotic dark red-staining piece visible, in which many leucocytes had accumulated. Shinya used dogs for his experiments and in transplanting the tissue he made a longitudinal split in the perineurium of the sciatic nerve, through which a piece of muscle was transferred into the middle of the nerve substance; the perineurium was afterwards sewed up with human hair to prevent the extrusion of nerve substance and of the transplant.

Concerning the methods by which the cross-striated muscle fibers regenerate, different views have been expressed. Some authors state that the regeneration occurs through outgrowth of cells of an embryonal type which later produce muscle tissue, while others maintain that it takes place by means of terminal and lateral bud formation in the cut muscle fibers. According to Krause[9] the nuclei of the old muscle fibers increase and around these the muscle cells form. Saltykow, in his experiments, never observed a genuine budding of the old contractile substance into young fibers. Shinya concluded that the manner of formation of new muscle fibers differs in the auto- and homoiotransplants. In the former there were seen mainly single sarcoblasts and cellular chains of syncytial elements, while in the latter the multinuclear buds of muscle were predominant. According to this author, this difference is a morphological expression of the biochemical differences between individuals of the same species.

Experiments

In studying the problems above stated, we used rats in which we excised a piece of the xyphoid cartilage, together with a part of the cross-striated muscle attached to it, and then transplanted it either into a subcutaneous abdominal pocket of the same rat (autotransplant) or of another unrelated rat (homoiotransplant). Rats from different breeds were used to ensure lack of relationship in case of homoiotransplantation. In every experiment each animal received both an auto- and a homoiotransplant in two different subcutaneous pockets, from which the pieces were removed after varying periods ranging from two days to one hundred eighteen days. In the main our observations were as follows:

Two-Day Autotransplant: The muscle fibers are almost everywhere degenerating, most of them having undergone necrosis. A few of the fibers still have cross-striations. Many polymorphonuclear leucocytes are invading and destroying the muscle. Small blood vessels are sometimes seen among the muscle fibers.

Two-Day Homoiotransplant: The appearance of the muscle is similar to that seen in the two-day autotransplant. Perhaps the fibers are slightly more fibrillar and granular in the homoiotransplant, but this difference is probably without significance.

Four-Day Autotransplant: The muscle, for the most part, is broken up and degenerated. Cross-striations can still be seen in a few degenerating fibers; the large majority of the nuclei are pyknotic. Peripherally, the muscle tissue is being replaced by fibroblasts, round cells and a few small blood vessels. However, in this area there are a few small slender muscle spindles which are multinucleated, the nuclei being vesicular. There are also present in the periphery, small circular remnants of muscle tissue cells containing much cytoplasm and showing multiple nuclei. Thus we find in this piece, associated with degenerative processes in the muscle fibers, proliferation of muscle nuclei.

Lymphocytes are numerous in the periphery and they have invaded and destroyed the muscle fibers in that region. Centrally, although the fibers are almost everywhere degenerated, there is very little lymphocytic infiltration.

Four-Day Homoiotransplant: Most of the muscle appears normal, although occasionally a necrotic fiber is seen. A few of the muscle

nuclei are pyknotic. There is here, little or no evidence of regeneration such as was seen in the four-day autotransplant. Peripherally there is a slight increase in the number of lymphocytes and fibroblasts.

Six-Day Autotransplant: In this piece there is almost everywhere very marked proliferation of the muscle nuclei. The transplant is made up chiefly of long slender muscle spindles which stain bluish pink and contain numerous vesicular nuclei. Very faint cross-striations can be seen in many of the spindles. Nucleoli are present in a number of the muscle nuclei. In a few of the muscle spindles the nuclei are clumped together at one end. Peripherally, many remnants of muscle tissue, small in size, round in shape and containing multiple vesicular nuclei are present. There are a moderate number of lymphocytes and connective tissue cells scattered diffusely between the spindles, invading and replacing some of them. Small blood vessels are also seen among the lymphocytes and connective tissue cells. No budding off of new muscle fibers from the old can be observed.

Six-Day Homoiotransplant: A great part of this transplant is made up of small, circular, multinucleated muscle remnants. There are seen also a number of slender pink-staining muscle spindles containing multiple large vesicular nuclei. However, the muscle spindles do not appear to be as numerous here as in the autotransplant, nor is the degree of nuclear proliferation as intense. No mitoses can be found. Very faint cross-striations are present in a few of the multinucleated spindles. Lymphocytes are seen almost everywhere in the transplant, the infiltration with these cells being much greater here than in the autotransplant. Many of the lymphocytes have, among them, large vesicular nuclei which are surrounded by only a very small amount of cytoplasm. There are, furthermore, scattered fibroblasts as well as small blood vessels throughout the transplant.

Ten-Day Autotransplant: At one end of the transplant is an area in which are seen moderately long, pink-staining muscle spindles which are multinucleated, the nuclei being situated, not in the center as formerly, but chiefly at the periphery of the spindles. In previous transplants the nuclei in the spindles were more numerous and vesicular and a larger number of them were situated centrally. Thus there is an indication that the muscle fibers are again attaining their

mature state; a large number correspondingly show very distinct and regular cross-striations. In the periphery of the transplant many muscle remnants are present and some round cells, as well as fibroblasts, are invading the muscle tissue leaving many muscle nuclei surrounded by small amounts of cytoplasm. Small blood vessels are also numerous in this area.

Ten-Day Homoiotransplant: The muscle is largely degenerated. In the central part of the transplant there are a few thick degenerated muscle fibers which still have distinct cross-striations. These fibers appear mature and represent, probably, the originally transplanted fibers which now have undergone degeneration. At the ends of several muscle fibers there are a number of large pyknotic nuclei. Very few round cells can be seen in this region. However, around this area and toward the periphery the muscle consists of degenerated remnants which are multinucleated and everywhere surrounded or invaded by lymphocytes. Here the lymphocytic reaction is marked. Lymph vessels filled with lymphocytes, as well as numerous small blood vessels, are found between many of the muscle remnants. Peripherally, a number of nuclei with very little cytoplasm about them lie among the round cells. The nuclei are, as a rule, not as large or as distinctly vesicular as those in the autotransplant, although some proliferation is apparently taking place. In addition to the lymphocytes, connective tissue cells are rather numerous in this area.

Fifteen-Day Autotransplant: There are present in this transplant, well formed slender muscle fibers with distinct and regular cross-striations. Very little degeneration of the fibers is seen. The muscle nuclei are still increased in number, but are not as numerous as in the earlier transplants; also they are in general smaller, more elongated and situated nearer the periphery of the fibers than the nuclei of earlier muscle pieces. There is almost no lymphocytic invasion, while a few very small blood vessels are seen between some of the fibers. Thus, the muscle now consists almost entirely of long slender fibers with good cross-striations staining normally, and showing still an increase of muscle nuclei. Nowhere can there be seen a true, new formation of muscle fibers with an outgrowth into an area which had originally been free of muscle tissue.

Fifteen-Day Homoiotransplant: The transplant is almost completely degenerated; it is surrounded by a connective tissue capsule

containing numerous round cells. Near the capsule, a few muscle remnants containing several peripherally placed nuclei are present, but centrally almost no muscle tissue is seen, it being replaced chiefly by blood vessels and a small number of round cells and connective tissue. However, some regeneration does take place, as is indicated by the appearance near the periphery of a very few small slender muscle fibers which are multinucleated, the nuclei being situated chiefly at the ends or the periphery of the fibers.

Twenty-Day Autotransplant: The muscle here has practically the same appearance as in the fifteen-day autotransplant. The fibers are well formed and slender. They also stain well and have distinct and regular cross-striations. They appear a little more mature than at fifteen days, the nuclei being less numerous, peripherally placed, spindle-shaped and more darkly staining. Small capillaries again are seen between the fibers while very few lymphocytes are present in the muscle. There is some sign of degeneration in a few areas at the periphery of the transplant where the fibers stain paler, appear granular and have no distinct, regular cross-striations.

Twenty-Day Homoiotransplant: In this piece the muscle fibers which occur along the side of the transplanted cartilage have been almost completely destroyed and replaced by round cells, connective tissue cells and small blood vessels. Only in a few places an occasional muscle remnant containing a few pyknotic nuclei is seen. Extending peripherally from the area first described is a group of large thick degenerated muscle fibers which are surrounded by a ring of round cells, connective tissue cells and blood vessels, together with occasional small degenerated muscle remnants. These large muscle fibers contain only a small number of round cells and appear to be the remains of the original transplanted muscle.

Twenty-Eight-Day Autotransplant: The muscle fibers as a rule have cross-striations and are rather long and slender. The nuclei are small, are not increased in number and are situated at the periphery. In some places, especially at the periphery of the transplant, the muscle tissue is degenerated, the fibers losing their stain and appearing granular and fibrillar. A very few of them are completely surrounded by connective tissue and are necrotic. Small blood vessels are scattered throughout the muscle tissue. No lymphocytic invasion of the muscle can be seen and while connective tissue is present about all of the muscle tissue, it does not invade the fibers. There are practically no lymphocytes in the connective tissue.

On the whole, the muscle fibers although somewhat irregular and degenerated in a few places seem to be mature and in good condition.

Thirty-Two-Day Homoiotransplant: To one side of the cartilage, there is a very dense area of lymphocytes which have almost completely destroyed the transplanted muscle in this area. The round cell reaction is extremely intense. In addition lymph vessels are packed with lymphocytes. In the middle of this area of round cells, and also approaching one end of the cartilage are seen rather numerous, very small and slender pale pink-staining muscle fibers which are spindle-shaped and which contain multiple, elongated nuclei at their periphery. Well defined cross-striations are present in a number of these fibers, which are well preserved in spite of the intense lymphocytic invasion about them. In one place in the periphery may be seen a number of small degenerated muscle remnants among the round cells. In this specimen, thus, the round cell reaction has become intense and has destroyed the greater part of the muscle; however, there is, even here, still a small amount of regeneration taking place.

Forty-Day Autotransplant: At one end of the cartilage transplanted muscle tissue is found. The fibers, on the whole, appear normal, stain well and have cross-striations. In the periphery a few of them are granular or show a fibrillar structure. The muscle nuclei are slender, elongated, stain darkly and are not increased in number. There is no lymphocytic invasion between or in the muscle fibers.

Forty-Day Homoiotransplant: The appearance of this transplant is very similar to that of the thirty-two-day homoiotransplant. The lymphocytic reaction is intense and masses of lymphocytes have replaced almost completely the transplanted muscle. In one area, at the periphery of the transplant, a number of muscle remnants are being invaded by round cells and connective tissue cells. In another area, likewise in the periphery, there are present a small number of short slender multinucleated muscle spindles, indicating that some regeneration is still taking place. However, no cross-striations are present in these spindles, which thus differ from those seen in the thirty-two-day homoiotransplant; here also, round cells and small blood vessels are invading and destroying the muscle spindles.

Fifty-Day Autotransplant: The transplanted muscle is represented

by a number of slender pink-staining fibers, which have distinct and regular cross-striations. The muscle nuclei are somewhat larger than normal, are multiple and situated at the periphery of the fibers. The muscle appears normal in every respect, except that the fibers are comparatively small and slender and contain an increased number of rather large nuclei. Fibroblasts are numerous and are invading and destroying many of the muscle fibers. Practically no lymphocytes are present.

Fifty-Day Homoiotransplant: On one side of the cartilage is an area containing many lymphocytes, fibroblasts and a few small blood vessels, which almost completely replace the transplanted muscle tissue. Occasionally, small, oval, pale pink muscle remnants which sometimes contain a few nuclei, are seen. These are the only traces of muscle tissue which remain. No evidence of muscle regeneration is seen.

Seventy-Day Autotransplant: The muscle fibers as a rule are small, but appear to be living. Many of them are somewhat irregular in outline. There is no lymphocytic invasion of the muscle; fat tissue is present between a number of the fibers.

Seventy-Day Homoiotransplant: The lymphocytic reaction in this piece is extremely intense, the lymphocytes taking up practically the whole of the transplant. Very few traces of muscle fibers remain. Occasionally some muscle nuclei lying within very small granular muscle remnants are seen, grouped together among the lymphocytes. Fibroblasts are rather numerous.

Eighty-Six-Day Autotransplant: The transplanted muscle has practically the same appearance as that of the seventy-day autotransplant.

Eighty-Six-Day Homoiotransplant: At one side of the cartilage there is an area of connective tissue in which there are several dense collections of lymphocytes; occasionally there is a lymph vessel full of lymphocytes. The round cells have replaced about all of the transplanted muscle. However, a few vesicular muscle nuclei are seen within very small slender spindle-shaped fibers which are lying among the lymphocytes. In some places a few degenerated fibrillar muscle remnants having no cross-striations are present.

One-Hundred-and-Eighteen-Day Autotransplant: The transplanted muscle fibers are living and well preserved at this stage. The large majority of them stain well and have well defined, regular, cross-

striations. They are of normal thickness, and appear in almost every respect like normal, mature muscle tissue. Some of the fibers, however, contain small chains of nuclei varying in number from three to eight, situated usually near the periphery. In some places, especially in the periphery, the fibers are small and granular and often show some invasion by a few connective tissue cells and lymphocytes. Small blood vessels are rather numerous in this region. The connective tissue cells and, to a less extent, the lymphocytes are somewhat increased in this autotransplant. In several one-hundred-and-eighteen-day autotransplants, no muscle tissue could be found, this being possibly due to the fact that the part of the piece containing muscle was not obtained for examination.

One-Hundred-and-Eighteen-Day Homoiotransplant: Almost no muscle tissue can be seen in this transplant, although at one end a very few small degenerated remnants are still present among the lymphocytes, which are very numerous in this region and have replaced the muscle. In other places, only connective tissue containing a few small blood vessels and scattered lymphocytes is observed.

Conclusions

In the autotransplants, during the first few days following the transplantation, the muscle is invaded by polymorphonuclear leucocytes and the greater part of it becomes necrotic. However, as early as four days after the operation there is found some proliferation of muscle nuclei within preserved fibers. At six days, we note a marked proliferation of muscle nuclei with the formation of long slender muscle spindles, many of which are again developing cross-striations. The number of leucocytes is decreased. The spindles subsequently take on a more mature appearance, the cross-striations becoming more definite and numerous, the nuclei decreasing in number and assuming a more nearly peripheral situation. At fifteen days the muscle consists chiefly of long slender fibers which contain good cross-striations, but which still show an increase in the number of muscle nuclei. Lymphocytes, which at earlier periods were present in small numbers, have at this time almost completely disappeared. At twenty-eight days, the muscle, except for slight signs of degeneration in a few areas, appears mature and normal in almost every respect. This is also the case at forty days, the muscle having reached

its greatest development at this stage. At fifty days there is noticeable much fibroblastic invasion of the muscle fibers, which are comparatively small, contain an increased number of nuclei but which have definite, regular cross-striations. At seventy and eighty-six days the muscle is still living and also at one hundred and eighteen days, at which period the fibers are in very good condition and look normal, except that in some of them there are a few small chains of nuclei and that there is also some increase in the connective tissue cells and lymphocytes. It should be mentioned, however, that not in all experiments was muscle seen at one hundred and eighteen days.

In the homoiotransplants at two and four days, the appearance of the muscle is similar to that of the autotransplants, but almost no evidence of nuclear proliferation is present in the four-day transplant, in contrast to the corresponding autotransplant. At six days the lymphocytic reaction is becoming distinct, although it is not yet very marked. The degree of regeneration, as shown by the number and appearance of the muscle nuclei, as well as of the spindles, is less in the homoiotransplant. At ten days the lymphocytic reaction increases in intensity and the muscle fibers undergo degeneration, with the consequent formation of many muscle remnants. There is still a slight proliferation of muscle nuclei at this stage. The lymphocytic invasion of the muscle and its destruction increase. At thirty-two days the lymphocytic reaction has become so intense that the muscle has been almost completely replaced by such collections of small round cells. However, among the round cells there can be seen a few small multinucleated muscle spindles with well marked cross-striations, indicating that slight regeneration may still be proceeding or that regenerated cells are surviving. The lymphocytic invasion continues to increase, with connective tissue cells also playing a part, and at fifty days only a few muscle remnants remain. At seventy days the round cell reaction has reached its height and now, as well as somewhat later, only a very few degenerated muscle remnants remain. Finally, at one hundred and eighteen days, practically no muscle tissue can be found, it having been replaced by lymphocytes, connective tissue and a few small blood vessels. Thus, it is seen that in the homoiotransplants regeneration takes place in a similar manner to the autotransplants; but the regeneration is less intense. Furthermore,

the degenerative processes due to the lymphocytes and, to a less extent, to the connective tissue, are much more pronounced in the homoiotransplants.

The differences between auto- and homoiotransplantation of cross-striated muscle tissue described in our experiments are in accordance with the results obtained by Loeb and his co-workers in the case of other tissues and also, in a general way, with the subsequent work of Shinya in the case of cross-striated muscle. However, Shinya, as has previously been mentioned, assumes that there exists a difference in the manner of regeneration between auto- and homoiotransplants, inasmuch as, in the former, not only muscle spindles develop, but also independent myoblasts appear at the edge of the wound, whereas in the latter only muscle spindles were seen. No such differences between these two types of transplants were seen in our experiments. Furthermore, in Shinya's experiments the muscle tissue of the autotransplants was replaced by connective tissue as early as thirty-five days after operation, while in our experiments good transplanted living muscle tissue was found as late as one hundred and eighteen days after transplantation, this being the longest period at which autotransplants were examined. In the homoiotransplants, Shinya found complete necrosis at twenty-seven days, while in our experiments a few multinucleated muscle spindles were present as late as forty days. Saltykow carried out only autotransplantations of cross-striated muscle and his description of the latter process is similar to ours; however, in contrast to our findings, he reported practically a complete disappearance of the cross-striated muscle fibers at the beginning of the fourth month.

Our experiments prove that after autotransplantation striated muscle can survive in the host at least for a period of four months without functioning. How much longer autotransplanted muscle could thus be preserved in the rat, we do not know. In certain places we found some indication that there was an increased activity of fibroblasts among the transplanted muscle fibers in the later periods. This may be taken as an indication that even in autotransplants some destruction takes place in the course of time.

As to the homoiotransplants, their injury and destruction are evidently due to two factors. Primarily, the regeneration and perhaps also the preservation in the first period following transplanta-

tion, are less in the homoiotransplants than in the autotransplants. This indicates a direct injury of the transplant by homoiotoxins, a conclusion which is in accordance with the observations of Hesselberg [10] and Loeb in the case of the unstriated muscle tissue of the uterus; and of Loeb in the case of the placentoma formation in the transplanted uterine tissue. In the second place, the lymphocytes and also the connective tissue take an active part in the destruction of the homoiotransplanted muscle tissue while they leave intact the autotransplanted muscle. In this respect striated muscle tissue behaves, after transplantation, like all the other tissues so far transplanted into other individuals of the same species.

There remains still one point to be discussed; namely, the evident lack of extensive regeneration, if we use this term in its strict sense, as indicating an outgrowth of tissue into a defect, leading to a filling in of the defect. As far as we can make out such an outgrowth does not take place to any noticeable extent; instead, we find a reformation, through growth processes, of a part of the degenerated muscle. We have, therefore, to deal in the case of transplantation of striated muscle tissue with a process in some respects akin to that which we find in compensatory hypertrophy, as for instance, in the liver, rather than with a regeneration such as takes place in the case of the epidermis.

Summary

1. After autotransplantation of striated muscle tissue in the rat, well preserved muscle tissue was found as late as one hundred and eighteen days after transplantation, the further fate of the muscle tissue not having been studied yet.

2. After homoiotransplantation, there is less regeneration of the muscle tissue in the first period after transplantation, and much more degeneration due to invasion by lymphocytes and, to a less extent, by connective tissue at subsequent periods. As a result of these conditions, homoiotransplanted muscle disappears much earlier than autotransplanted muscle, no preserved tissue being present at fifty days.

It is therefore probable that in addition to the action of the lymphocytes, the homoiotoxins may injure the muscle tissue directly, diminishing the regenerative process and increasing the subsequent degeneration.

3. Nowhere was there seen a true outgrowth of muscle fibers, either in continuity with the preformed transplanted muscle or by budding. The old muscle tissue, after a primary degeneration of its major portion, undergoes regenerative processes, consisting in an increase of its sarcoplasm and a proliferation of its nuclei; subsequently there is a differentiation of the sarcoplasm into typical striated muscle, in which cross-striations are again formed. In the course of time the nuclear proliferation becomes less active; the nuclei decrease in number and become situated peripherally within the fibers, while the fibers as such increase in size, until finally there are again present almost normal muscle fibers. This growth process is, in certain respects, comparable to compensatory hypertrophy or hyperplasia rather than to regeneration in the strict sense.

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INTRACRANIAL CHORDOBLASTOMA *

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In 1846 R. Virchow noted on the surface of the clivus Blumenbachii a small slimy excrescence attached to a defect in the bone (Fig. 1). He spoke of it at the Würtzburger physikalisch-medizinischen Gesellschaft on February 24, 1856, and in 1857 gave it the name of *ecchondrosis physaliphora*. In the meantime the same structure had been described by Luschka (1856), but its true nature was first pointed out by Müller in 1858.

Müller showed from the study of human material that the ecchondrosis physaliphora was a persistent remnant of the notochord, but his work was not accepted as final and the nature of the malformation was disputed until the publication by Ribbert (1895) of his experiments on animals which proved conclusively the notochordal origin. In 1894 Ribbert suggested the name *chordoma* which has since been employed.

It was soon found that similar formations might develop all along the spinal canal, particularly at the sacral extremity. The records of the cases described have been collected in the reviews of Coenen (1925), of Corsy and Surmont (1927), and of Stewart and Morin (1926). The ecchondrosis physaliphora of the clivus Blumenbachii was soon discovered to be a fairly frequent malformation, being found in 2 per cent of necropsies by Ribbert and in 1.5 per cent by Stewart and Morin. Interest in it soon declined, although occasional reports occur in the recent journals (*cf.* Ajello, 1927), but was revived by the publication of cases in which it had reached a size suffi-

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cient to cause symptoms of intracranial tumor. The first record of this kind is that of Klebs (1864), in which is contained the account of a middle-aged man, said to have died of tetany. At necropsy was found a cloudy-white tumor attached to the clivus and measuring 1.5 by 2 by 1.8 cm. in diameter. Klebs was not sure that the tumor had caused the symptoms from which his patient suffered but undoubted instances have since been published to the number of about thirty-two, namely; the cases of Grahl (1903), Seiffer (1905), Fischer and Steiner (1907), Linck (1909), Frenkel and Bassal (1910), Jelliffe and Larkin (1911), Eitel (1911), Spiess (1911), Wegelin (1911), Hässner (1912), Kotzareff (1918), Argaud (1918–19), Daland (1919), Fabricius-Møller (1919), Hellmann (1921), Swars (1921), Lemke (1922), Mathias (1923), Hirsch (1923), Burrow and Stewart (1923), Arauz and Podestà (1923), André-Thomas and Jumentié (1923), Grossmann (1923), Filippini (1924), Eckel and Jacobs (1925), Citelli (1927), Castex and Camauër (1927), Camauër, Llambias and Mortola (1927), Sedláčkoviá-Šikl (1927), Argaud and Clermont (1928) and of Loebell (1928).

Details concerning most of the above tumors are readily available in the reviews of Stewart (1922) and of Stewart and Morin (1926). We may abstract the others briefly as follows:

Hirsch (1923). Mentioned in discussion of Grossman's case that he had a similar nasopharyngeal tumor, on which he operated by splitting the palate. It recurred a year later. No other details given.

Arauz and Podestà (1923). Female, 17. For twelve years difficulty of respiration and nasal speech. Tumor in left nasopharynx. X-ray showed lesion of axis. Paralysis of left external rectus. Vomiting but no headache. Developed slight hemiparesis. Biopsy; death fifteen days later. Illustrations show typical chordoma.

Filippini (1924). Female, 36. For seven years nasal voice. For three years difficulty of swallowing. Lately failing vision in right eye and finally disturbance of equilibrium. Right eye blind. Nystagmus on looking to right and left. Dysphagia. Paresis of right facial and of soft palate. Necropsy disclosed miliary tuberculosis. Tumor size of mandarin orange on clivus.

Citelli (1927). Male, 18. For one year difficulty in breathing. Bleeding from nose. Tumor filling nasopharynx. Biopsy; abundant hemorrhage. Partial removal. Recurred two years later.

Castex and Camauër (1927). Female, 20. Sleepiness and rapid oscillation of right eye. Later headache and vomiting. Dysarthria. Neuralgia in both jaws. Dysphagia. Paralysis of tongue. Right ptosis. Dilatation of right pupil. Paralysis right vocal cord; also right trapezius. Paresis both sixth nerves. Exaggeration tendon reflexes on left side. Died of pneumonia. Translucent tumor extending from optic chiasm to foramen magnum.

CAMAUËR, LLAMBIAS, AND MORTOLA (1927). Male, age not given. Transitory attack of loss of consciousness with weakness of left foot and double vision. Paresis right twelfth nerve. Left hemiparesis. No choking of optic discs. Died of pneumonia after a second attack. Small gelatinous tumor on clivus.

SEDLÁČKOVIÁ-ŠIKL (1927). Mentioned by Podlaha and Pavlicka. Details not available.

ARGAUD AND CLERMONT (1928). Female, 48. Small tumor of nasopharynx removed. Recurred several months later. Removed by trans-sinuso-facial route. Examination showed tumor to be chordoma. Said to have had "no ocular troubles."

LOEBELL (1928). Male, 62. Difficulty in breathing through left nares. Feeling of pressure in left ear, dizziness and faintness. Mass in left nasopharynx. Left third, sixth, and eighth palsy. X-ray showed sella turcica destroyed and erosion of petrous bone. Operated through antrum. Treated with radium. Recurred after one and a half years. Paralysis of both 3rd, 5th, 6th, 9th, and 10th nerves. Death. No necropsy.

To these records we wish to add two others, believing that in the case of so rare a tumor the publication of isolated instances in as much detail as possible is not only justifiable but necessary if a satisfactory general view of their structure, clinical course and treatment is to be obtained.

CASE REPORTS

Our first experience with these tumors occurred in 1928 when in examining the specimen from a supposed case of pituitary tumor we found the typical physaliferous cells. The record follows:

Case I. Intracranial Chordoblastoma with Symptoms of a Suprasellar Tumor. Transfrontal Exploration with Removal of what was taken for a Suprasellar Adenoma. Recovery.

On Jan. 4, 1928, Dr. A. M. Goldman of New York referred to the Peter Bent Brigham Hospital (Surg. No. 30402) a married Jewess, aged 34, complaining of headache and failing vision.

The family history was irrelevant. She began to menstruate at 14 years of age; was married at 20, and became pregnant but aborted after two months. A unilateral ovarectomy was performed. Since then her catamenia had been regular and normal. For about eighteen years she had recurrent headaches of gradually increasing frequency consisting of a dull ache above the inner angle of the left eye. During the past year she had gained about 20 pounds in weight. For the past three months her vision had been rapidly failing. Her physician stated that she had recently become somewhat childish and irritable and that her memory had been poor.

Examination on admission showed an alert and coöperative woman. There was found a bilateral primary optic atrophy (visual acuity 20/100 on either side), bitemporal hemianopsia, slight obesity (wt., 70 Kg.; ht., 1.58m), blood pressure slightly low (108/68), a slightly subnormal basal metabolic rate (−12), normal skin and distribution of hair, normal sella turcica, and normal urinary

considerable bleeding, necessitating packing of the cavity. The patient pulled the drains from his nostrils and died of hemorrhage during the night.

A complete necropsy was made. The general examination was not remarkable. The brain was removed after fixation *in situ* with the sphenoidal bone attached. There was a large friable mass of tumor about 5 cm. in diameter lying between the infundibulum and pons and pressing the peduncles forcibly upward (Fig. 3), compressing and elongating the 3rd, 4th, and 6th cranial nerves. The left trigeminal nerve was also compressed. The hypophysis was flattened against the anterior surface of the growth.

The tumor was preserved in 10 per cent formalin. Blocks were embedded in paraffin and sections stained with hematoxylin and eosin, and with hematoxylin mucicarmine-metanil yellow; others were impregnated by Perdrau's method.

Microscopic Description: The tumor consists essentially of large vacuolated cells arranged in cords and masses, with a varying amount of intercellular material (Figs. 4 and 5).

The cells are usually vacuolated in such a way as to leave a small amount of granular cytoplasm around the nucleus, but in many the vacuole lies excentrically. The nuclei vary enormously in size and shape; some are vacuolated. No mitoses are found.

When stained with mucicarmine and metanil yellow the cytoplasm appears yellow but the intercellular substance is bright red. The latter is usually amorphous or granular but in a few places is denser and homogeneous, having the appearance of cartilage. In these areas it has a reddish violet color in the Perdrau preparations; elsewhere it is not impregnated. In the cartilaginous regions occur granular deposits of calcium (Fig. 6).

A few thin-walled blood vessels are present, surrounded by cuffs of lymphocytes.

Comment: From the microscopic description and position of the tumor there can be no doubt of its nature. In Dr. Cushing's monograph the tumor is said to consist of "embryonic cartilage containing a few bone-cells with myxomatous and connective tissue." It was interpreted at the time by Dr. Welch as a "mixed tumor from a congenital anlage." According to our own examination the tumor consists of unmistakable notochordal tissue with a great amount of mucoid intercellular stroma in which scattered islands of cartilage are differentiating. The only bone we found was in the inferior and outer parts of the growth and might have been a fragment of invaded cranium. There were, however, scattered granular deposits of calcium such as were found by Hässner (1912).

Cartilage normally forms from the notochord during regeneration (Barfurth) and during embryonic development in lower vertebrates, perhaps also in mammals and man (Salvi, 1899). It should not be surprising, therefore, to find cartilage differentiating in chordomas.

Discussion

Gross Appearance: These tumors are almost invariably described as having a grossly nodular but smooth surface, of a milky-white color. The cut surface is semitranslucent resembling that of cartilage, but is of softer, often jelly-like, consistency. There are cysts filled with a slimy material resembling mucus. Other tumors are described as reddish or brownish, probably due to interstitial hemorrhage, although they are usually poorly vascularized and bleed only slightly when incised.

These growths arise in the base of the sphenoidal bone and may project either upward into the intracranial cavity from the clivus Blumenbachii or downward into the nasopharynx through the craniopharyngeal foramen. In several cases there was both an intracranial and an intrapharyngeal extension (Wegelin, Spiess, Mathias, Linck, Hellmann). There are a few cases recorded in which the tumor seems to have been confined to the nasopharynx (Argaud and Clermont, Grossmann, Fabricius-Møller), but in two of these there is no record of a neurological examination and in no instance has the absence of intracranial extension been proved by necropsy.

The tumor may extend into the sphenoidal sinuses, the orbits of the eyes or even into the neck (Daland, 1919) and regional veins (Wegelin 1912).

Microscopic Structure: The microscopic appearance of these tumors is remarkably uniform. The most characteristic feature is of course the vacuolated or physaliferous cells. It has been abundantly proved (Franco 1922, Grossmann 1923, Mathias 1921, Podlaha and Pavlica 1928) that the vacuoles are filled with glycogen. Since its demonstration demands fixation of the tissue in absolute alcohol we were unable to find it in our material. But we were able to show the presence of intercellular mucin, very abundant in Case I, less so in Case II. Sometimes the cells are widely separated by the mucin, causing a superficial resemblance to a chondroma; in others they are so closely packed that the tumor resembles a small-celled

carcinoma (Hellmann 1921). The cells are usually arranged in groups or cords.

The nature of the nuclear vacuolization, which was frequently seen in the tumor of our Case II, is still disputed. These vacuoles are said also to contain glycogen (Stewart, 1922).

Diagnosis: The microscopic diagnosis is fairly easy, but the clinical diagnosis presents difficulties. Kotzareff has said that the diagnosis during life is impossible. This is obviously not true of those tumors with a nasopharyngeal extension. Several such cases have been diagnosed by biopsy (Mathias, Loebell, Linck, Hirsch, Hellmann, Grossmann, Fabricius-Møller, Argaud and Clermont).

But the diagnosis of purely intracranial chordomas is certainly not easy. The symptoms, as one might suppose, consist essentially of palsies of the cranial nerves, from the third to twelfth pairs. In such cases of multiple palsies the possibility of a chordoma must be remembered. By extension forward to the optic chiasm a bitemporal hemianopsia may result (Spiess, our Case I); by distortion of the pons and peduncles pyramidal symptoms arise; by extension backward cerebellar symptoms are produced, and by increasing size symptoms of intracranial tension appear. However, although headache is common, choking of the optic discs is relatively rare and late in development. It is mentioned in six of the thirty-five cases; in two of them it was very slight. A more frequent complaint is pain on movement of the neck, doubtless due to extension of the tumor into the foramen magnum.

In a few cases (Spiess, Loebell, Kotzareff) destruction of the base of the skull has been demonstrated by the X-rays. It is possible that with better technique this destruction could be more often seen. In cases of basal tumor we suggest that a stereoscopic examination be made with the plates under the patient's chin and the rays coming from the parietal region. In this way the clivus Blumenbachii can be clearly outlined and small defects readily detected.

The principal tumors to be differentiated would seem to be the carcinoma of nasopharyngeal origin which has invaded the intracranial cavity, the hypophysial carcinoma, the meningioma of the cavum Mecklii, the neurinoma of the acoustic nerve, the tumors of the hypophysial duct, and perhaps a rare pearly tumor. Of these the acoustic tumors and the tumors of the hypophysial duct have such a definite chronology and symptomatology that the confusion need

rarely arise; the pearly tumors do not erode the bone; and the palsies caused by meningiomas of the cavum Mecklii are unilateral. But the carcinomas of nasopharyngeal or hypophysial origin are more difficult to differentiate. We know little of the latter tumors. It is to be hoped that the forthcoming study of Oljenick will aid in their recognition. Nasopharyngeal carcinomas can of course often be diagnosed by examination of a specimen removed from the original tumor in the nasopharynx.

In conclusion we would say that in any case of multiple palsies of the cranial nerves the possibility of a chordoma should be kept in mind; an examination of the nasopharynx or a proper roentgenological study might aid in establishing the correct pathological diagnosis.

Treatment: Study of specimens at necropsy gives little hope that these tumors can ever be completely removed. Vincent is said to have removed the tumor from the clivus in Argaud's first case. The condition of the patient afterward is not mentioned; one may well imagine it. Attempts to reach the tumor by transfrontal (Cushing), temporal (Kotzareff), suboccipital (André-Thomas and Jumentié), transnasal (Spiess) or transmaxillary (Loebell) operations have been equally unsuccessful. The nasopharyngeal extensions have been removed and treated by radium (Loebell) or X-ray (Daland) but have invariably recurred after a short interval.

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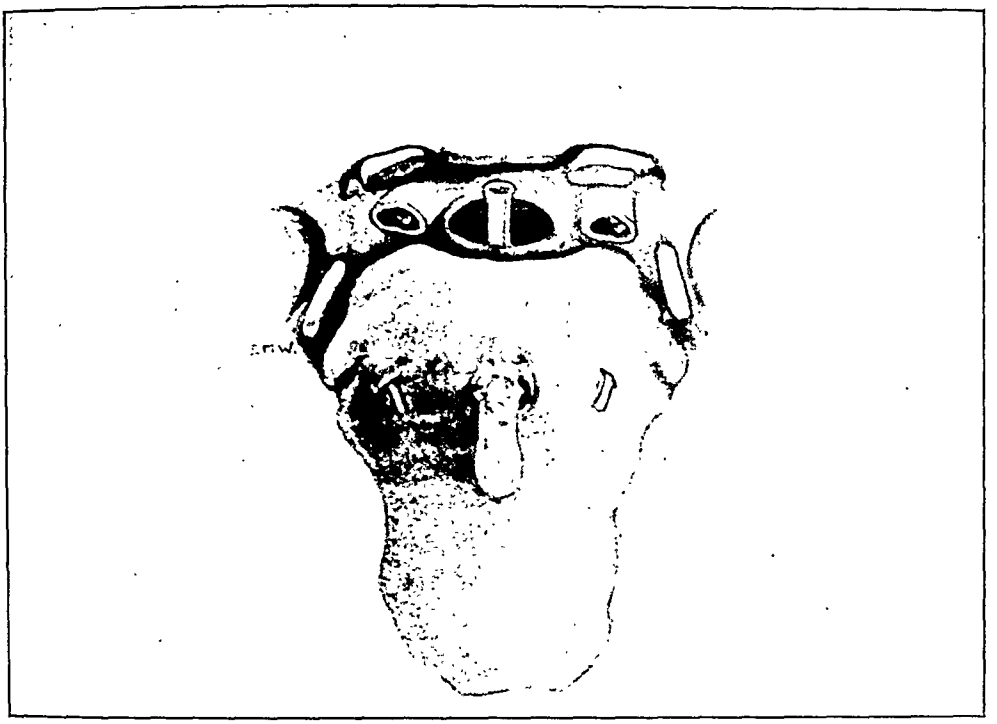
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DESCRIPTION OF PLATES

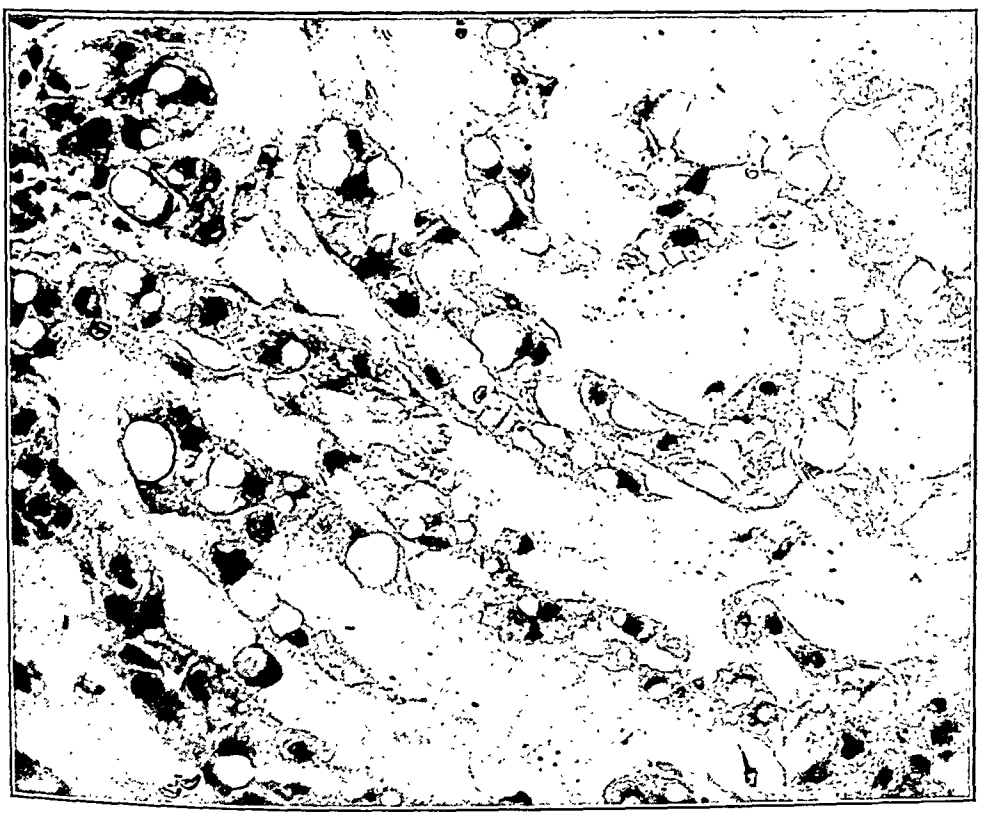
PLATE 88

FIG. 1. Ecchondrosis physaliphora (After Burrow and Stewart).

FIG. 2. Case I. Photomicrograph of tumor. Methylene blue-eosin. Note the cords of vacuolated cells separated by mucinoid material. × 300.



1



2

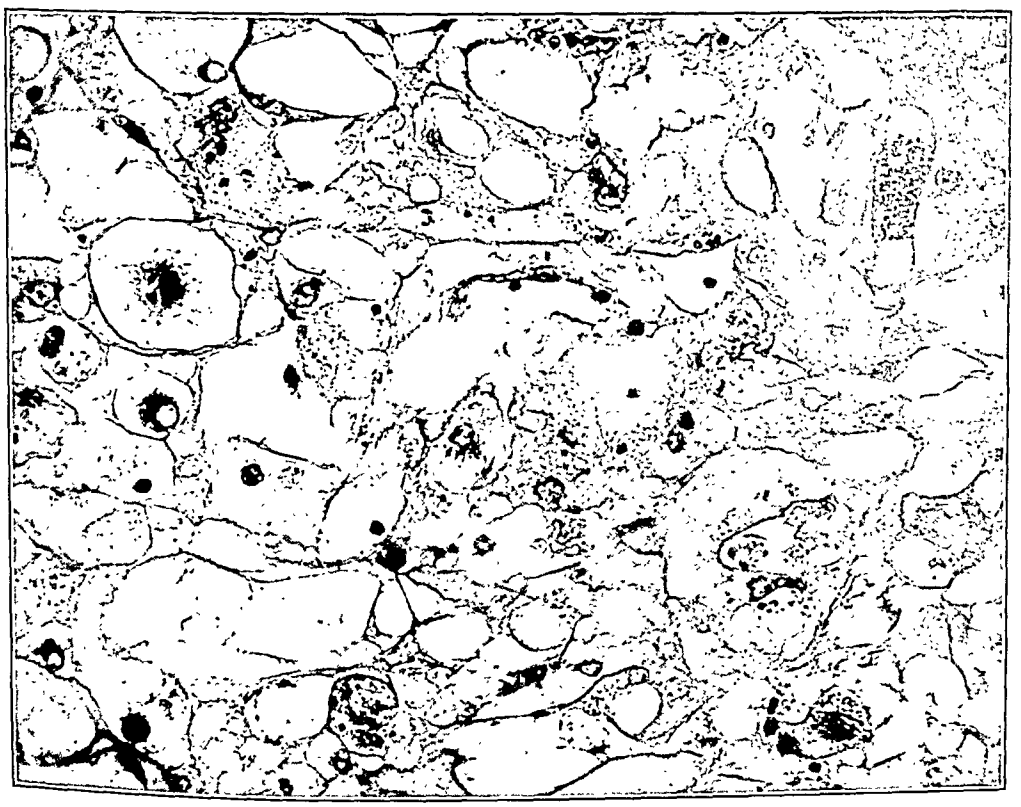
PLATE 80

FIG. 3. Case II. Median section of brain with tumor in place. Note its position between the infundibulum and pons.

FIG. 4. Case II. Photomicrograph of tumor. Iron-hematoxylin. Vacuoles are present both in the cytoplasm and nuclei of the cells. × 300.



3

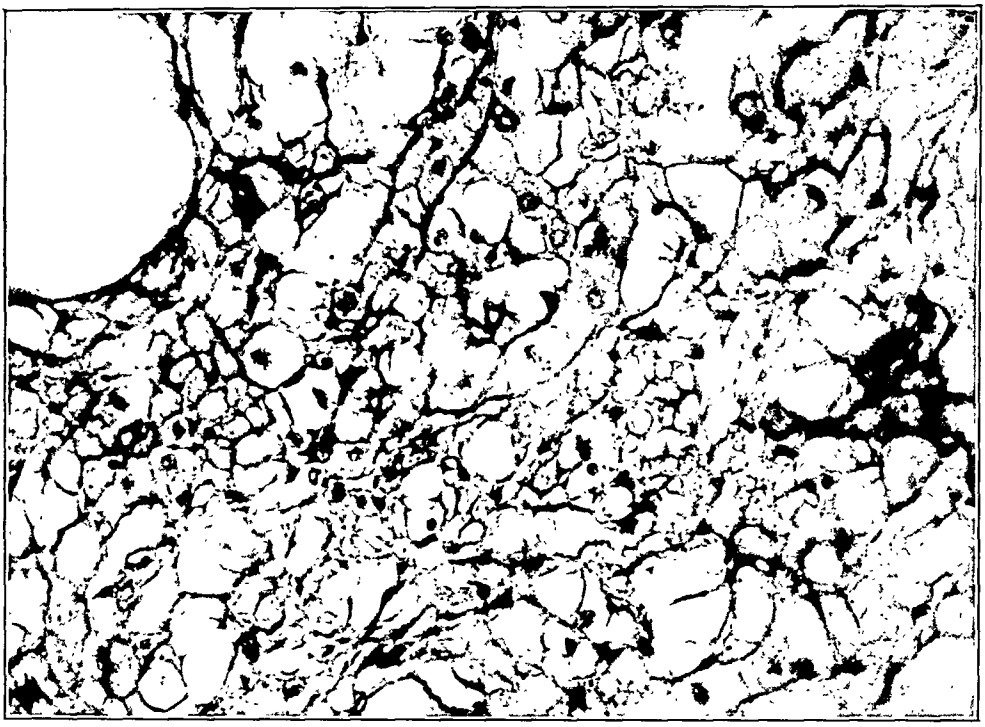


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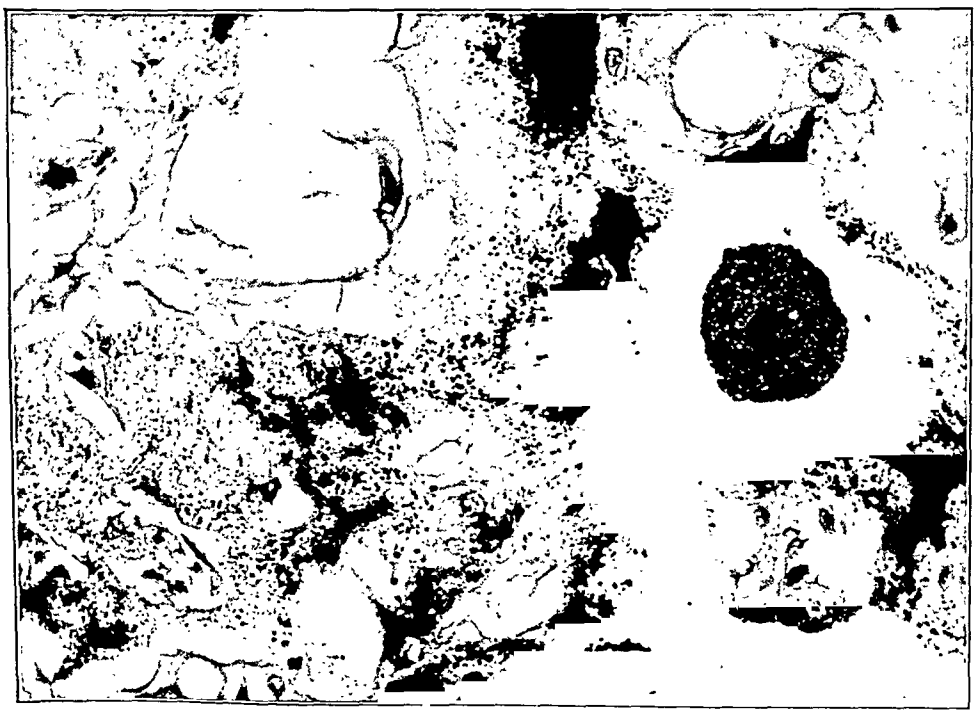
PLATE 90

FIG. 5. Case II. Cords of vacuolated cells are seen. Mucicarmine-metanil yellow. × 325.

FIG. 6. Case II. Calcium salts being deposited in the mucoid stroma. Hematoxylin and eosin. × 300.



5



6

INTRAVITAL STAINING OF MICROGLIA WITH TRYPAN BLUE*

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It is commonly accepted that the intravital selection of the benzidine dyes from colloidal solution is characteristic of the cells of the so-called reticulo-endothelial system (Kiyono,[1] Aschoff[2]). Those to whom the name "reticulo-endothelial system" is objectionable will still admit the existence of a macrophage system with vital staining properties in the body whether these macrophages are labelled clasmatocytes, endothelial cells, Marchand's adventitial cells, or collectively as histiocytes. Excluding the central nervous system these macrophages have been extensively studied; the reader is referred to Aschoff's [3] paper and to Maximow [4] for reviews of the work and the relevant literature.

The macrophages of the central nervous system have as yet been imperfectly studied with the vital stain technique from the point of view of their relationship to, and possible origin from, glia. This is doubtless due, in part, to the recent development of the metallic impregnation methods of the Spanish school by which the different types of glia can be distinguished. Although vital staining has been applied to the brain, the procedure has not yet been combined with subsequent staining of sections by the silver carbonate methods of Hortega. Thus Macklin and Macklin [5] were able to produce a striking coloration of the tissues about cerebral puncture wounds in rats, using trypan blue intravitally, and they described the appearance of granules of dye in the macrophages in such areas. They thought that some of the neuroglia cells also contained blue granules, but without special staining methods they were unable to trace the relationship of their macrophages to the glia. More recently Kubie [6] applied Sabin's supravital technique to the same problem with the object of elucidating the relationship of microglia to the phagocytes of the brain. He demonstrated the presence of lymphocytes and clasmatocytes in the perivascular tissues of normal rabbits and guinea pigs by the supravital method, and intravitally in a rabbit

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that had received repeated small injections of trypan blue in the subarachnoid space. He could reach no conclusions concerning the part played by microglia in phagocytosis. His inability to do so may be attributed to his apparent omission of the silver impregnation methods for demonstrating microglia. Moreover, he does not appear to have made use of the microglial reaction round cerebral puncture wounds, although he produced such punctures in six of his twenty-two animals.

The vivid staining reaction obtained by Macklin and Macklin together with their suggestive observations on the deposition of trypan blue granules in the macrophages about puncture wounds, encouraged the view that a repetition of their procedure combined with specific staining of the microglia would throw some light on this relationship.

The microglial reaction around a sterile puncture wound of the cerebrum has been described and figured by Hortega [7] and by Hortega and Penfield.[8] Their observations have recently been repeated by Carmichael.[9] The striking accumulation of microglia cells in the periphery of the wound and the gradual transition between the normal resting form of the cell with its numerous branched processes and the rounded-up, phagocytic forms, known as compound granular corpuscles or *Gitterzellen*, are clearly demonstrated by these authors. The minute cytological changes involved in this transition have recently been described by Cone.[10]

Experimental

The rabbit was used for this work on account of the ease with which microglia can be demonstrated in this animal. The cerebral puncture was performed under ether anesthesia by tapping through the skull a three-inch nail the tip of which had been filed down to a fine tapering point. The nail was flamed before use but was allowed to cool down before being brought into contact with the tissues. The skin had been previously prepared by cutting the hair close and applying tincture of iodine. In no instance was there any evidence of bacterial infection.

A preliminary microscopic study of the reaction about the wound obtained in this manner showed that the optimum time for observing transitional forms of microglia lay between the second and fourth days after the operation. The reaction obtained at such a

stage is seen in Figs. 7 and 8.* It was therefore decided to observe the effects of intravital staining at this optimum period. Incidentally Macklin and Macklin found that the best intravital staining of the macrophages was also demonstrated within the same period, an observation that added point to the investigation.

Cerebral puncture was therefore repeated in conjunction with intravital staining. The trypan blue was administered by the intraperitoneal route following the directions given by Foot.[11] The sample of dye used was obtained from the National Aniline and Chemical Co. The dose used at first was 10 cc. of a 1 per cent solution of the dye in distilled water per kilo of body weight, but it was felt that the histological results obtained left room for improvement. The dose was then doubled without evidence of any toxic effect upon the animal, and there is little doubt that the dye could be pushed further if an even stronger reaction were desired. The first dose was given three hours before the brain puncture was performed and was then repeated at twenty-four hourly intervals. At the end of three or four days the animal was killed with chloroform, and the brain immediately transfused through the carotid artery with formalin ammonium bromide. Later the brain was removed and transferred in suitable blocks to a bottle of the same fixative. Sections were cut at 15 microns on the freezing microtome a few hours later, or on the following day, and were stained for microglia by Hortega's ammoniacal silver carbonate method (Hortega,[12] see also Penfield[13]). In using the method it was found advisable to leave the sections in the silver bath for less than thirty seconds, for a heavy impregnation is liable to obscure the granules of trypan blue, especially in the early transitional forms of microglia. Also prolonged toning in gold will change the blue of the dye to a purplish tint that is indistinguishable from the surrounding cytoplasm; for this reason the time in the gold bath was not longer than ten minutes.

Description of Results

The rabbits showed rapid absorption of the dye from the peritoneal cavity. In the course of two hours after the first injection the skin, conjunctivae and gums assumed a distinct blue tinge, and the

* These two figures are reproduced here with the kind permission of Dr. Penfield; they have already been published by Hortega and Penfield.[8]

color became more brilliant as time progressed. The brain wound was therefore produced at a time when the trypan blue had access to the site of injury. After twenty-four hours the rabbits excreted bright blue urine. There was no evidence that the dye exerted any toxic action upon the animal in these experiments.

On postmortem examination there was intense deep blue staining of the abdominal and thoracic viscera and of the soft tissues generally. Where the smaller dose of trypan blue had been employed the cerebral puncture wounds showed a faint blue halo only around the central clot. When the larger dose had been administered there was a bright blue halo, the macroscopic effect resembling that figured by Macklin and Macklin in their experiments on the rat. The rest of the cortex appeared normal. There was a brilliant blue staining of the choroid plexuses, and the leptomeninges showed many fine dots and streaks of blue, especially in the deeper sulci.

Microscopically there is a microglial reaction about the wound, of the type that has been described by Hortega and Penfield (Figs. 7 and 8). The track of the wound is occupied by recent blood clot in which a few *Gitterzellen* containing numerous granules of trypan blue are scattered. In the borders of the wound every type of transition can be traced between normal resting microglia and rounded *Gitterzellen*. On examination with high powers of the microscope discrete granules of trypan blue of variable size can be detected in the cytoplasm of all these transitional types (Figs. 2 to 6 inclusive), but, as might be expected, the blue is most abundant in the typical *Gitterzellen* and is relatively difficult to find in microglia cells that have undergone little change (Fig. 2). Almost all the trypan blue in the tissues about the wound can be accounted for by this intravital selection of the dye by microglia and its derivatives.

Granules of dye are also conspicuous in ameboid or spindle-shaped cells lying in the adventitia of the vessels in the neighborhood of the wound. These cells correspond without doubt to the clasmatocytes demonstrated supravitally by Kubie. They can be identified also as Marchand's adventitial cells. There are also granules of dye in round and spindle cells in the leptomeninges. Occasionally a few cells containing granules of trypan blue, which are not impregnated with silver when treated by Hortega's method for microglia, are found in the brain tissue adjacent to the wound. These cells are probably monocytes that have migrated from the blood stream.

They are relatively scarce in comparison with microglia cells in the early stages of reaction in these wounds and assume a more conspicuous part in the histological picture in the later stages, for example in wounds examined one week after the injury. Attempts to demonstrate granules of dye in the astrocytes by Hortega's silver carbonate method were unsuccessful.

In the rabbits used there was no evidence of that form of focal chronic encephalitis that is apt to appear spontaneously in laboratory animals of this species.

Discussion

The presence of numerous granules of trypan blue in the compound granular corpuscles, or *Gitterzellen*, and in their antecedent ameboid forms calls for little comment as an isolated observation. The origin of compound granular corpuscles, however, has been a subject of controversy. According to Alzheimer [14] these cells arise either from mesodermal tissues or from neuroglia. Metz and Spatz [15] consider that they are derived from microglia, but regard microglia as a form of neuroglia and hence of ectodermal origin. Nissl [16] traced them to a mesodermal source, and it is believed in many quarters at the present time, on the basis of Hortega's work, that microglia furnishes the greatest number of these phagocytic cells in pathological conditions of the brain, and that microglia is of mesodermal origin.

Whether all the *Gitterzellen* are derived from microglia or whether some of them may or may not arise more directly from other mesodermal sources (for example from the adventitial clasmatocytes) is outside the scope of this paper. The demonstration of granules of trypan blue in all transitional forms between typical microglia and typical *Gitterzellen* serves not only as additional evidence of their direct relationship, but also places microglia on an unequivocal footing as a part of the reticulo-endothelial system. It may be objected that microglia is precluded from this category because one cannot demonstrate granules of dye in the cytoplasm when the cell is in its resting form (Fig. 1). It is recognized, however (Maximow [4]), that the ability of macrophages in general to absorb the colloidal dyes is linked up with their phagocytic or ameboid activity. It is necessary, therefore, to employ a stimulus such as a simple aseptic puncture wound to activate these cells, and hence to demonstrate their true nature.

Conclusions

1. The intravital staining of microglia with trypan blue is demonstrated, using aseptic puncture wounds of the cerebrum as a stimulus to phagocytic activity.

2. It is claimed that such intravital staining identifies microglia with the rest of the reticulo-endothelial system of the body.

3. These conclusions offer further support to Hortega's contention that microglia is a mesodermal element.

4. There is no evidence of the formation of *Gitterzellen* from neuroglia or that neuroglia is able to take a vital stain.

My best thanks are due to Dr. Penfield and to Dr. Cone for much guidance and advice.

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DESCRIPTION OF PLATES

PLATE 91

Intravital staining of microglia in border of stab wound of cerebrum of rabbit four days after operation. Twenty cc. of 1 per cent trypan blue (5 doses) intraperitoneally.

FIG. 1. Normal resting microglia cell in hippocampus.

FIG. 2. Early transition. Scanty granules of trypan blue in cytoplasm.

FIG. 3. Two cells showing later stage with granules of trypan blue in swollen cytoplasm. The left-hand cell shows typical spinous branches of microglia at upper extremity.

FIG. 4. Later change toward ameboid form. Trypan blue granules more abundant and processes almost lost.

FIG. 5. Late transition stage; ameboid form of microglia.

FIG. 6. Typical compound granular corpuscle or *Gitterzell*.

Camera lucida drawings. × 900.

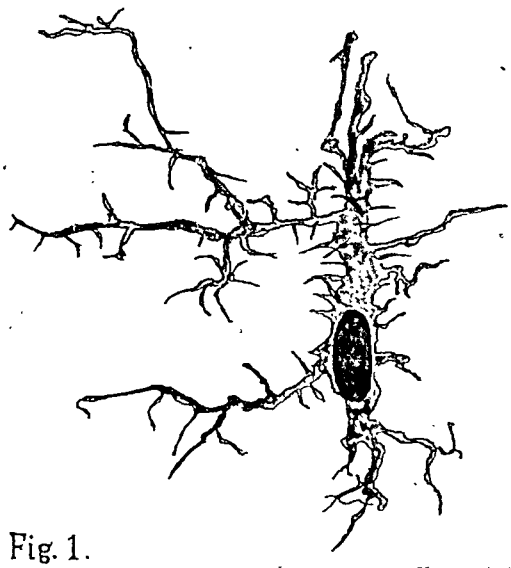


Fig. 1.

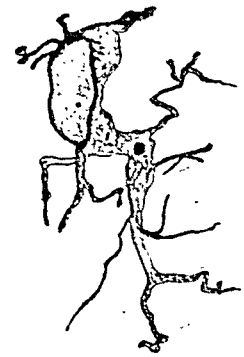


Fig. 2.



Fig. 3.

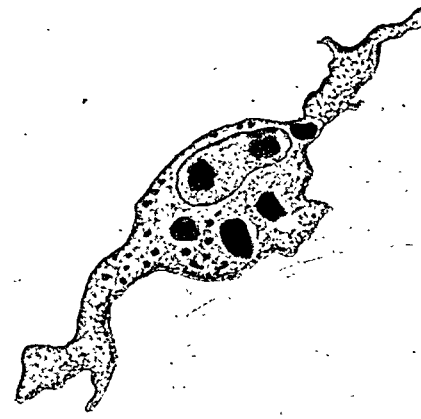


Fig. 4.

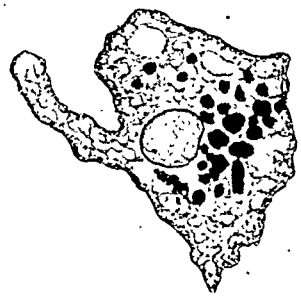


Fig. 5.



Fig. 6.

D.S.R.

PLATE 02

FIG. 7. Compound granular corpuscle forms of microglia at the border of a stab wound (T) made in the rabbit's brain two days previously. Silver carbonate microglia method.

FIG. 8. Same case as Fig. 7, higher power. Farther from the tract (T) there were seen transition stages between the branching form and this ameboid form of microglia. Silver carbonate microglia method.

(These photomicrographs are reproduced with the kind permission of Dr. Penfield.)



7



8

ABNORMALITY OF THE CEREBRUM AND LEPTOMENINGES SIMULATING AN INTRACRANIAL TUMOR *

R. C. BUCKLEY, M.D. AND E. M. DEERY, M.D.

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Localized gross abnormalities in the development of the brain are rarely met with in a neurosurgical clinic and may well be mistaken for brain tumors. Recently, the only large verified abnormality of the cerebrum observed in this clinic was considered as a tumor from its appearance. Had this lesion been exposed at operation it would undoubtedly have been considered as a tumor. Further, it is possible that if a piece of the tissue had been removed at operation the immediate diagnosis of an astrocytoma would have been made.

There are many interesting findings brought out in the study of the lesion to be described, but the purpose of this report is to present it from the standpoint of its clinical and gross resemblance to a tumor and to mention certain histological findings in the undeveloped meninges of interest in relation to the origin of the meninges.

REPORT OF CASE

P. B. B. H. Surg. No. 32960. Case of suspected brain tumor in an infant. Convulsions and asymmetrical enlargement of head developed soon after birth. Ventriculograms and examination pointed to cerebral tumor. No operation. Death from bronchopneumonia. Autopsy.

Clinical History: Arnold S., aged six months, was brought to the Children's Hospital of Boston on December 19, 1928, for observation. A history taken there by Dr. L. K. Diamond brought out the following facts. The infant was a second child of healthy parents. Their first child is living and well. This baby was born after a rather difficult labor, but at birth showed no evidence of injury. It was noted that there were six toes on each foot. During the first few days of life the child appeared to act normally in every respect. At the end of the first week, however, it was apparent to all that its head was becoming abnormally large. At two weeks the first convulsive attack occurred. This consisted of flexion of all four limbs, rolling up of the eyes and deep labored breathing. The attack lasted from three to four minutes. Similar attacks followed daily from then on.

Physical examination showed an apathetic, poorly developed infant with a large occipital bulge to the head. The head measured 52.5 cm., the chest 42.5

* Received for publication June 7, 1929.

cm., and the abdomen 40 cm. The height was 61 cm. The superficial vessels of the scalp were dilated, and the cranial sutures and fontanelles were open. Eyes showed internal strabismus, but no downward displacement of the eyeballs. Liver edge, spleen and left kidney were palpable. There was a slight lordosis with marked scoliosis. Six toes were seen on each foot. Knee and ankle jerks were hyperactive.

The cerebrospinal fluid was under increased pressure, sterile, contained no globulin and no abnormal cells. The blood and spinal Wassermann, general blood, stool and urine examinations were negative. The results of ventriculograms reported by Dr. E. C. Vogt were as follows:

"The right ventricle appears dilated and definitely larger than the left with a constricted area posteriorly. Repeated studies showed the same marked asymmetry of lateral ventricles with what is evidently a soft tissue mass depressing the right ventricle posteriorly from without."

The infant was considered a brain tumor suspect and transferred to the surgical service of this hospital December 31, 1928.

Examination here was essentially the same except that no epigastric or abdominal reflexes were obtained. It was felt that the deep reflexes of the legs were greater on the left. Beginning bronchopneumonia of both lower lobes was found.

The first impression of the case was in accord with a possible right cerebral tumor. Further studies were withheld, and all efforts directed to the bronchopneumonia, which, however, proved fatal in two days. Permission to examine the head only, was obtained.

Necropsy: The head was asymmetrically enlarged by a distinct bulge of the entire right side. The cranial sutures were widely separated. The thin cranial cap was removed with difficulty because of the adherent dura mater. When the dura was reflected there was apparent a large firm tumor-like mass in the right frontoparietal lobe (Fig. 1). *In situ*, this mass was of hard rubber consistency. The falx cerebri was pushed to the left. The brain weighed 980 gm.

The blood vessels over the base of the brain and cerebral hemispheres, elsewhere than the tumor mass, appeared normal grossly. Over the tumor area there were seen several large confluent venous channels lying in incomplete sulci (Fig. 1). There was also a decrease in the number of small superficial vessels over the same area as compared with the corresponding area of the normal appearing left side (Fig. 1).

The tumor-like mass replaced the superior half of the entire frontal lobe and extended back to the right paracentral region of the parietal lobe. Anteriorly the mass was sharply marked off from what remained of the frontal lobe. Laterally and posteriorly the lesion was less sharply demarcated, and, indeed, it was hard to tell where it ended in the gradual transition to the normal sulci and gyri. An-

teriorly the mass was like one huge gyrus with only psuedo-sulci, shallow umbilicated areas and local thickenings of the leptomeninges to interrupt its smoothness.

The leptomeninges over this area only were abnormal. They were firmly adherent to the cortex and were studded with multiple, irregular local translucent, gray-white thickenings of from 1 to 11 mm. in any diameter (Fig. 2). These areas were not related to blood vessels although occasionally blood vessels appeared superficially imbedded in them. They did not resemble cerebral herniae but suggested in the gross either local inflammatory reactions or masses of tumor cells in the subarachnoid spaces.

The remainder of the brain and a small portion of the cervical cord appeared normal externally and in comparison with several other brains of infants of the same age.

After fixation a series of coronal sections demonstrated the error in believing this lesion to be a tumor; for there was a gross abnormality corresponding with the lesion seen externally as well as an asymmetrical hydrocephalus involving the lateral and third ventricles. The many sections through the abnormal area showed it to be composed of a translucent white zone of 1 to 3 mm. in depth, beneath which there was a thickened gray matter and a decreased amount of white matter (Fig. 3). About the dilated right lateral ventricle the white matter was never more than 4 mm. in thickness. Both the gray and white matter appeared much firmer than the corresponding tissue in the left hemisphere.

The asymmetrical hydrocephalus involved the lateral and third ventricles. The capacity of the right lateral ventricle was about twice that of the left lateral ventricle. The foramina of Monro were dilated, equal, and measured 1 cm. in diameter. The aqueduct of Sylvius, the fourth ventricle and the basal cysternae appeared of normal capacity. The foramina of Magendie and Luschka were not demonstrated. The only abnormality of the ependyma consisted of a fenestrated dam-like thin membrane 1 cm. in height arising from the posterior portion of the floor of the anterior horn of the right lateral ventricle (Fig. 3). The choroid plexus, basal ganglia, cerebellum, pons, and optic chiasm appeared normal.

Microscopic Examination: Blocks of tissue from corresponding areas on each side of the formalin-fixed brain were taken for study both by frozen and paraffin sections. Hematoyxlin and eosin, eosin-

methylene blue, phosphotungstic acid hematoxylin, Nissl's stain, Pal-Weigert's, and Loyez and Perdrau's stains were made on the paraffin sections. Gold chloride sublimate, Penfield's combined method for oligodendroglia and microglia, Bielschowsky's, and Scharlach R. stains were made on the frozen sections.

Only in the abnormal portion of the right frontal and parietal lobes were changes of the leptomeninges found. The outstanding lesions were: (1) the presence of nests of astrocytes in the subarachnoid space connected with the brain through the defect in the pia mater; (2) the marginal gliosis; and (3) the lack of, or a partial myelinization of the nerve fibers in the white matter with a resulting extensive gliosis. No abnormal changes of the blood vessels were noted other than that they were increased in number in the white matter and scarce in the cortical gray matter. The neuronal elements in every part of the brain appeared unchanged.

The nests of astrocytes made up what in the gross appeared as the translucent gray-white localized thickenings of the leptomeninges. In many sections a connection between the cortex and the islands of tissue in the subarachnoid space was seen (Fig. 6). This connection appeared as a narrow break in the pia mater. There was no streaming of astrocytes into this subarachnoid tissue as is seen in a small hernia or an adhesion formation following injury to the leptomeninges. Again, there was no increased vascularity or growth of blood vessels toward or from the meninges such as is seen in a reaction to injury. There were many protoplasmic, lesser numbers of fibrous astrocytes, few neuroglia fibrils and nests of what appeared to be astroblasts. The latter had, in places, definite relations to blood vessels. In some areas they were closely approximated to the outer wall (Figs. 4 and 5). In other areas they occurred in long clumps apparently unconnected with blood vessels. There was very little connective tissue reaction in the leptomeninges, and this was always about the islands of astrocytes (Fig. 6). The significance of the astrocytes in this position is not known. They are localized in the only gross abnormal part of the brain where there are small defects of the pia mater. Nerve cells, nerve fibers and microglia or oligodendroglia were not demonstrated in these areas in the leptomeninges.

The marginal gliosis was present only in the abnormal areas. It corresponded with the thin white cortical zone seen beneath the

leptomeninges in the gross. This gliosis was not seen at the site of the defect in the pia mater where the cortex and the subarachnoid nests are connected.

In the white matter of the involved areas, the usual closely packed nerve fibers were partially replaced by a dense gliosis, unmyelinated fibers and occasional small areas of hemosiderin deposits. There was no subependymal reaction.

The neuronal elements were normally arranged, and no abnormalities of these cells were observed.

Many unsuccessful attempts to stain microglia and oligodendroglia in the abnormal areas were made but they were not demonstrated. They were successfully stained in other parts of the right hemisphere and left hemisphere. The significance of the lack of demonstration of these elements cannot be discussed as they may well be present and not brought out due to personal errors in technique.

In no section of the brain was there found an abnormality of the blood vessels, cell reaction about the blood vessels or in the cerebral tissue other than the gliosis mentioned above. No large or atypical ganglion cells were seen.

Comment

This case is an example of a cerebral tumor suspect in an infant with a history of convulsions and the development of an asymmetrically enlarged head. The ventriculograms were interpreted as showing a tumor compressing the posterior horn of the right lateral ventricle. The patient was then transferred to this clinic for the purpose of making an exploratory craniotomy. However, an intervening respiratory infection proved too much, and death occurred shortly after admission.

At necropsy and before the brain was sectioned, the lesion was taken to be a tumor of some unusual sort. The error was obvious after sectioning the brain.

The reason for the lack of development of the right frontoparietal area was not found. As far as could be determined there was no abnormality of the larger cerebral arteries. In the absence of any evident block in the ventricular system the asymmetrical hydrocephalus could not be explained. The aqueduct of Sylvius was patent, and the fourth ventricle was of normal size.

Without the complete autopsy a classification other than a localized abnormality of development will not be attempted. At this point attention may be drawn to the associated polydactylism. The defects of myelinization and the marginal gliosis only in the abnormal area are similar to the lesions seen in some forms of cerebral sclerosis. There are no subependymal areas of gliosis forming projections such as are so frequently described among the lesions found in cases of tuberous sclerosis.

In addition to the similarity of the lesion to a tumor the multiple, local areas of incomplete development of the leptomeninges and the presence of nests of astroblasts and astrocytes in the subarachnoid spaces of these areas are of considerable interest. These nests of cells of the astrocyte type are unusual findings. The cells are stained specifically by gold chloride sublimate and show up well with the phosphotungstic acid hematoxylin preparations. Glia fibers are present in small amounts, and only very few fibrous astrocytes are seen. Microglia and oligodendroglia could not be demonstrated in the nests, and no nerve cells or nerve fibers were seen. The lesion was not suggestive of any stage of an inflammatory process. The nests of cells resembled somewhat those found by Wolbach [1] as multiple, scattered nests of astrocytes in the subarachnoid spaces of spinal cord. Wolbach made multiple serial sections and proved that there was no connection between these nests and the spinal cord. The nests of cells described here differ from those he described in that they are connected with the cortex and that by specific stains astroblasts and astrocytes are demonstrated. The presence of astroblasts suggests the formation of the astrocytes in the leptomeninges of this region. However, were there no connections of these nests of astrocytes to the cortex the suggestion that the astrocytes present in the leptomeninges were formed from ectodermal elements derived from the neural crest might be offered, not only to interpret their presence but also to support the view of Harvey and Burr [2] as to the origin of the leptomeninges.

Conclusions

A localized abnormality of the right cerebral hemisphere was found after death in the brain of an infant suspected during life of having a brain tumor. The lesion, when disclosed, was thought to be an unusual tumor until the brain came to be sectioned.

Had this lesion been exposed at operation it might easily have been mistaken for an enucleable tumor, and had a fragment of one of the localized thickenings of the meninges been taken for immediate diagnosis the tissue might well enough have been reported as a glioma.

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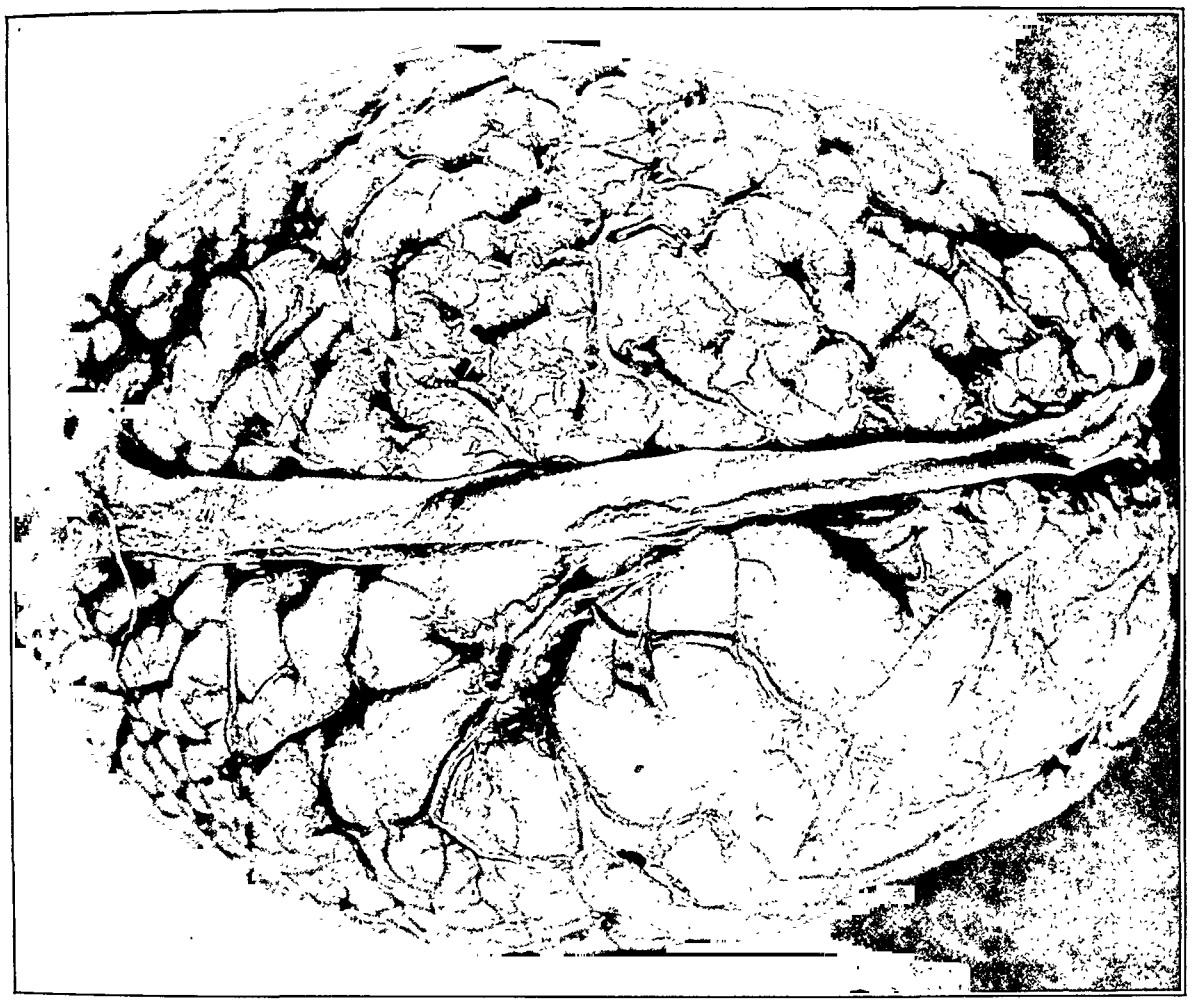
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DESCRIPTION OF PLATES

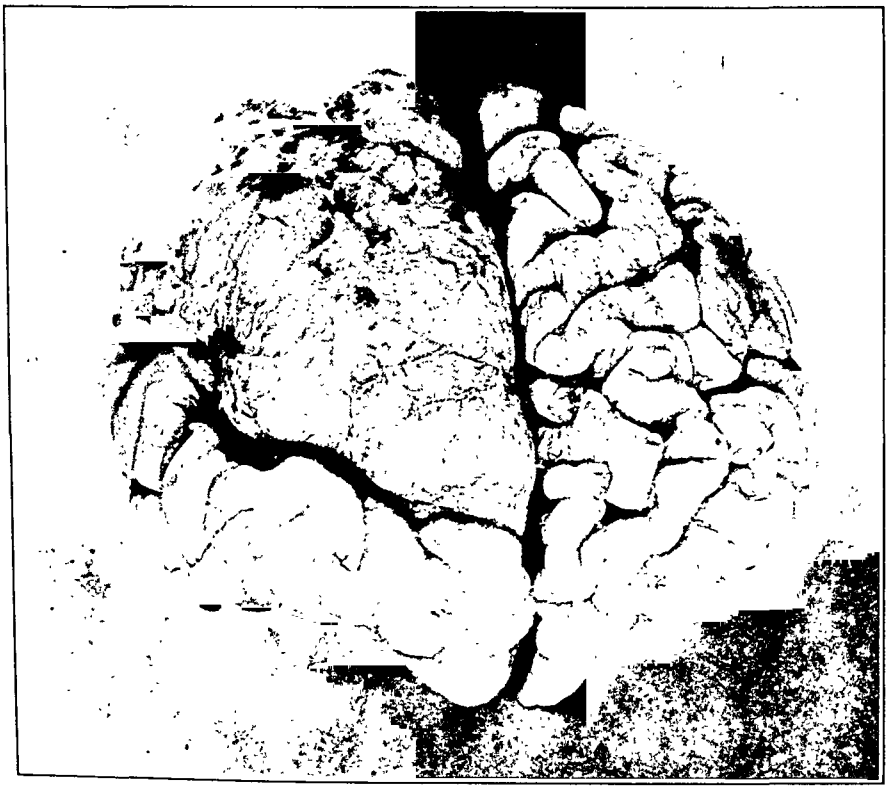
PLATE 93

FIG. 1. A view of the superior surface to show the abnormality of the right frontoparietal lobes. The macrogyri and large dilated blood vessels at the posterior portion of the abnormality are well brought out.

FIG. 2. A portion of the leptomeninges has been removed from the frontal poles to show better the contrast between the normal left side and the abnormal right side. Irregular areas of thickening of the leptomeninges are evident over the abnormal portion which appears to be enucleable.



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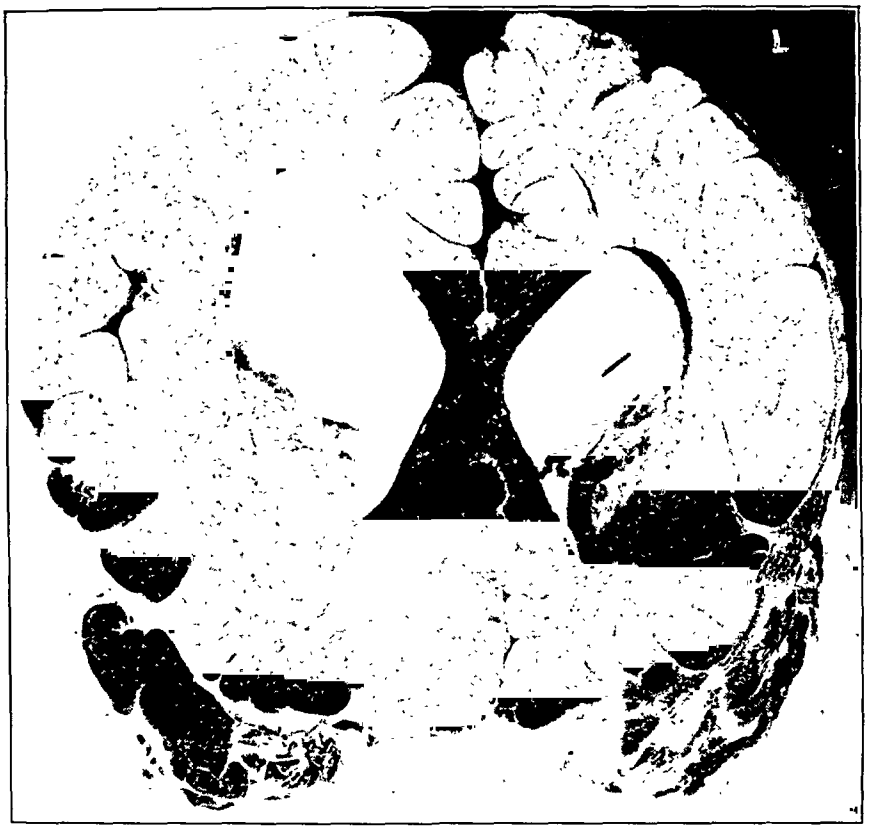
Buckley and Deery

Abnormality of Cerebrum and Leptomeninges

PLATE 94

FIG. 3. This section is taken from in front to show the dilated right ventricle with the dam-like structure within. The right hemispheres show well the abnormality of gyri and the gray-white marginal zone of gliosis. The apparent anomaly in the wall of the left ventricle was produced by postmortem inflation of the ventricles with air.

FIG. 4. A frozen section, stained with gold chloride sublimate, through a local thickening of the leptomeninges. The clusters of astrocytes stream along parallel with the cortex. There is no evidence of a pia mater. × 175.



3



4

Buckley and Deery

Abnormality of Cerebrum and Leptomeninges

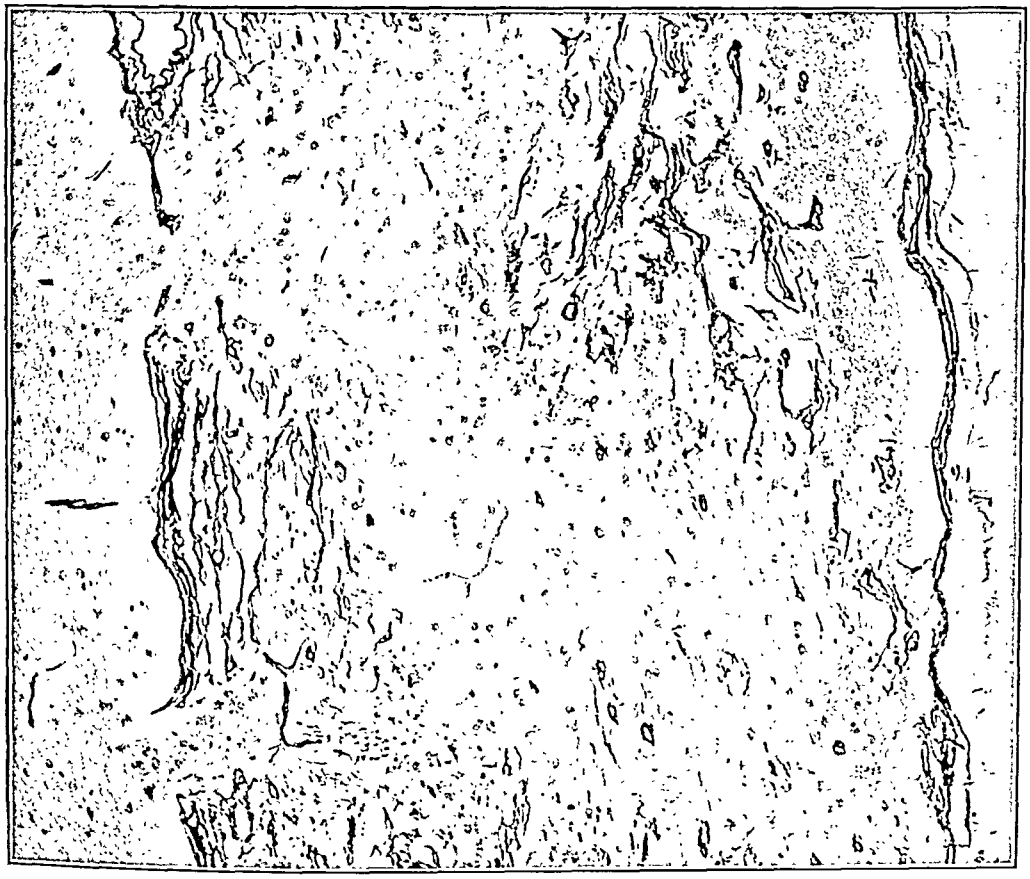
Plate 95

Fig. 5. Section through the thickened leptomeninges and cortex to show the connection between the two. (*a*) There are multiple nests of astrocytes (*b*) even near the under surface of the pia arachnoid. Many of the cells in these nests resemble astroblasts and are closely approximated to blood vessels (*c*). Gold chloride sublimate. × 175.

Fig. 6. Section through the leptomeninges and cortex stained by Perdrau's method to show the defects in the pia mater and the connections between the cortex and the glial tissue in the leptomeninges. The nuclei of the astrocytes are shown by this stain. There is no connective tissue shown in the areas of astrocytes in the leptomeninges. × 110.



5



6

TISSUE CULTURE STUDIES OF THE GLIOBLASTOMA MULTIFORME *

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As a part of the examination of each intracranial tumor verified at operation in this clinic, tissue culture preparations are made by the hanging drop method [1] in order, if possible, to further our knowledge of the histiogenesis of the tumors.

Growths have been observed in the cultures of certain of the gliomas, acoustic nerve tumors, metastatic epithelial tumors, adenomas of the hypophysis and ependymal tumors. However, the best results and the most consistent in type of growth are obtained from the operative specimens of the glioblastoma multiforme,† a rapidly growing invasive glioma of the cerebral hemispheres. This tumor is the most common type among the 496 verified and classified gliomas studied in this clinic. Up to February 1, 1929, there were 160 verified examples of this type of glioma. From ten to fifteen of these tumors are available in this clinic for study each year.

The purpose of this report is to present the results of the cultures made from the tissue of tumors diagnosed as glioblastoma multiforme, ten of which have been studied by the tissue culture method.

Of these ten separate tumors, five showed excellent growth *in vitro*, but no comparable growths were obtained from the remaining five although the same technique was used. Although many difficulties are met with in tissue culture preparations, it did seem that the lack of growth could be explained in some other manner. When the clinical histories and operative findings of the ten cases were studied, they were found to be similar to those of previously verified cases of the same type of glioma. But when histological studies of the paraffin sections were made there was found a considerable variation in cell type and arrangement. The histological sections of the five cases showing excellent growth in culture showed quite similar

* Received for publication June 7, 1929.

† These tumors were formerly termed spongioblastoma multiforme, but in accordance with the suggestion of Bailey and Cushing [2] the designation glioblastoma is now employed as more properly descriptive.

cell types and architecture. They were invasive, rapidly growing, with many areas of necrosis. The majority of the cells were those with a single large nucleus, a pyriform or fusiform-shaped body and either one or two processes. They resembled spongioblasts. Large multinucleated cells were frequently seen, but astrocytes were less common. Mitotic figures were abundant. The diagnosis accepted in each case was a glioma of the glioblastoma multiforme type. The histology of the remaining five tumors from which poor or no growths were obtained, varied. They were interpreted as rapidly growing gliomas with cells differentiating into astrocytes; probably of the glioblastoma multiforme type. That certain of these diagnoses were later changed is shown by the fact that one year postoperative the necropsy specimen of one tumor was considered as a typical astroblastoma. The predominating cell in these sections was of the protoplasmic astrocyte type with occasional giant cells and pyriform or fusiform cells with one or several processes.

Before considering the characteristics of the successful cultures the following history is presented. It will serve as an example of the known life history of any of the tumors that grew in culture as well as an illustration of the methods of examination of brain tumor tissue removed at operation.

Surg. No. 29667. Philip T., aged 42 years, dated the onset of his present illness to July 16, 1927, when a sudden twitching of his facial muscles occurred. One month later a left brachial monoplegia and signs of increased intracranial pressure developed. Two months from the onset of his illness he was admitted to this clinic, and a diagnosis of a rapidly growing glioma of the right parietal region was made — "most probably a glioblastoma multiforme." At operation, what was regarded by Dr. Harvey Cushing as grossly a typical spongioblastoma multiforme was partially removed by suction. During the operation a part of the tissue was sent to the laboratory for purposes of supravital preparations and tissue culture. A study of the immediate supravital preparations, made by Dr. L. S. Kubie, supported the gross diagnosis. The tissue was very cellular with the majority of the cells being interpreted as spongioblasts. The multinucleated cells and fibrils were less plentiful. Growths in many separate preparations of the same tissue studied by the supravital technique were observed over a period of ten days, subcultures being made. Morphologically, the growing cells resembled those seen in the supravital smears.

Within a week paraffin sections stained by the eosin-methylene blue and Mallory's phosphotungstic acid methods were available. The structure of the tumor was that of a rapidly growing glioma with many areas of necrosis and a varied type of cells with many mitoses, typical of the glioblastoma multiforme.

From any of the above studies a poor prognosis was offered. Extensive X-ray therapy was given. However, a recurrence of his symptoms was reported; and

within fourteen months from the onset of his symptoms death occurred at his home. A necropsy was not performed.

The tissue culture preparations from each of the five separate tumors diagnosed spongioblastoma multiforme were made within thirty minutes of the time of removal at the operation. The best preserved and most cellular appearing tissue was used. Observations of the cultures were made in a warm box adjacent to the incubator. Attempts at photomicrography of the living cells were in general unsuccessful and resulted in the loss of some preparations. So the preparations were fixed at intervals in 5 per cent formaldehyde, formalin ammonium bromide, and Zenker's solution. Photomicrographs were then made of the fixed, unstained and stained preparations. The fixed sections were stained with methylene blue, hematoxylin and eosin, gold chloride sublimate, and silver carbonate.

Remarkable similarities in the arrangement and type of the cells and the methods of cell division were seen in each of the growing preparations. These features will be taken up separately.

The pattern of growth is shown well in Figs. 1 and 2, where the suggestion of a rapidly growing, invasive type of tumor is evident. Such a cell growth has not been seen in the other cerebral tumor tissue cultures. Although in many preparations, individual cells were seen jutting out from the periphery of the explant within several hours of culturing, the growth in each culture was usually apparent from twelve to eighteen hours after planting. It is possible that some of these cells were mechanically displaced in culturing and then extended out. In addition to these definite cell bodies (Figs. 1, 2, 4, 6) extending out from the periphery, there were frequently seen small, rounded, finely granular anuclear bodies, varying in size, surrounded by a shining membrane, the whole being attached to the edge of the explant or to the end of a process coming out from the explant (Figs. 2, 3, 7). They were also found attached to cells and their processes out in the field of growing cells. Their significance is not known. They were not seen in the cellular portions of the paraffin sections of the same tumors. Actual cell formation from these bodies was not seen and they did not appear to be dying or dead cells.

Soon extension of the cells into the media was seen. The method of outgrowth, the connection of the cells and the type of cells were similar in the different cultures (Figs. 2, 3, 4). In some fields (Figs. 1, 4, 6) cells with fusiform bodies, a single nucleus and two long, taper-

ing processes predominated. The nuclei of these cells varied in position. At the edge of the explant where the cell body was pushing into the media and there was no process, the nucleus was seen at the advancing, blunted pole of the cell body. Among the growing cells the nuclei of the same cells were situated in the center or at one pole. The granules closely packed the cell bodies and extended out into the processes for varying lengths. Often there were local swellings in the processes caused by the accumulation of granules.

Connected by long, thin or thick processes with the above described cells there were many irregular, gigantic, multinucleated cells (Figs. 2, 4, 5). As many as seven nuclei have been counted in these large cell bodies. The method of formation of these large cells was not determined. A fusion of small cells to form a giant cell was not seen.

Occasionally cells different from the above grew out from the explant. These cells are the only type resembling astrocytes with the specific stains seen in any of the tissue cultures (Fig. 4). They have irregular bodies and a large, oval, vesicular, eccentrically placed nucleus. A nucleolus was not observed in these cells. They are made star-shaped by from 4 to 7 processes which extend out in any direction to be attached to a process of another cell of any of the above types. Fibrillae could not be demonstrated in their cell bodies or processes.

These various cell types are seen in the cultures fixed at from 24 to 144 hours after culture. In the few successful subcultures the pattern of growth and cell types resembled, in the living and fixed preparations, the original cultures. Just how long cultures of these tumors could be kept alive by this technique is not known. In the series the longest preparation was kept ten days. When it had extended to the vaseline ring on the cover slip it was fixed and stained.

Cell division by mitoses was commonly seen in the cultures and growths. Regular mitotic figures in the fusiform type of cells with a single nucleus were observed at the edge of the explant and out among the growing cells. In Fig. 3 the end result of mitosis is seen as the two separate cell bodies are dividing. Irregular mitoses were seen in the star-shaped and multinucleated cells (Figs. 4, 5). Wherever mitotic figures were present the cell processes were much thicker than in the apparently non-dividing cells. No other form of cell division was seen.

A fairly close resemblance in cell types was noted in comparing the living and fixed stained tissue cultures with the supravital preparations and the stained paraffin sections. The various cultured cells, as illustrated, were well impregnated by the gold chloride sublimate and the silver carbonate stains. The cells were less intensely stained by methylene blue and hematoxylin and eosin. Attempts at other and more complicated stains were unsuccessful.

From these observations, the most common type of cell growing in the cultures of the tumors diagnosed as glioblastoma multiforme is that with a fusiform or pyriform cell body, an oval nucleus, an occasional nucleolus, with a process extending out from one or both ends of the cell body. The granular cytoplasm of the cell body is continuous with the processes. These cells resemble very closely unipolar and bipolar cells, forming the most common cell in a successful culture of a medulloblastoma in which the cells appear to be differentiating into spongioblasts. The less commonly found cells were the multinucleated ones and those resembling astrocytes. The mitoses were of regular formation in the small and common type of cells.

Summary

Tissue culture preparations of ten intracranial gliomas removed at operation were made. The clinical histories and the gross structures of these tumors as seen at operation resembled previously verified examples of glioblastoma multiforme.

The cultures of five of these tumors showed excellent and uniformly similar growths. The cultures of the other five tumors presented none or very little growth.

The histological structure seen in the paraffin sections of the tumor tissue from which a growth was obtained was interpreted as similar to that of the glioblastoma multiforme. A different structure in the other five tumors made the diagnosis difficult. They were interpreted as a rapidly growing glioma with cells differentiating into astrocytes.

There was noted a striking similarity in the resemblance of the growing cells to those seen in fresh smears and stained paraffin sections of the same tumor tissue.

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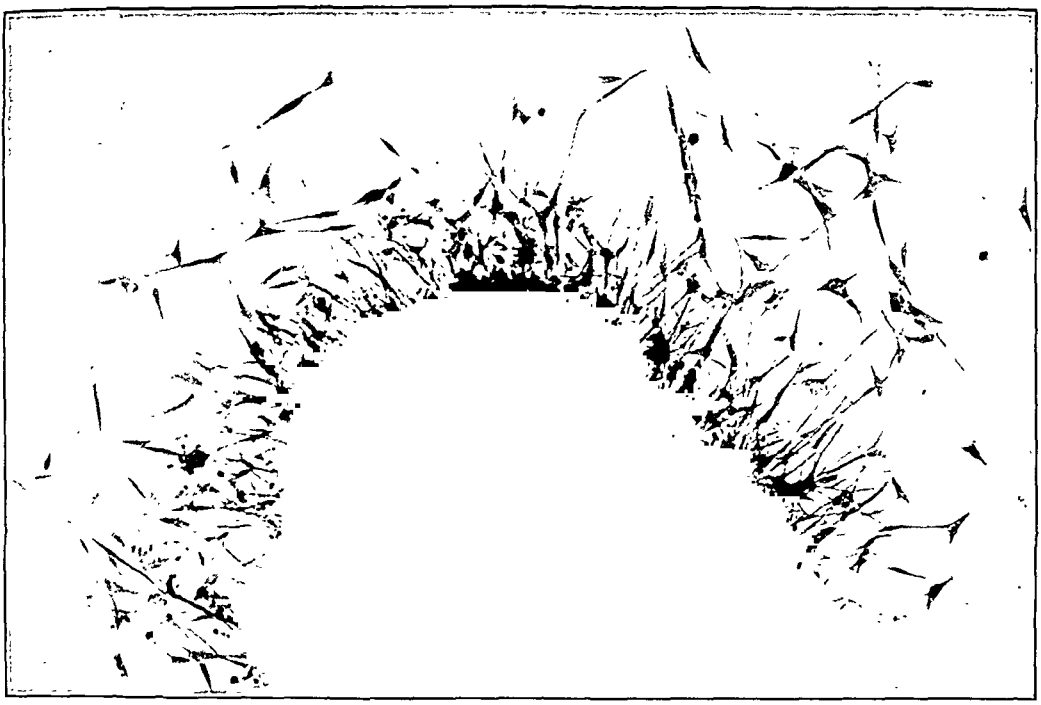
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DESCRIPTION OF PLATES

PLATE 96

FIG. 1. S 29667. Cell growth of a culture of glioblastoma multiforme fixed at ninety-six hours, the cells extending out to the edge of cover slip. Gold chloride sublimate. × 80.

FIG. 2. S 29667. Edge of explant of culture of glioblastoma multiforme at end of forty-eight hours, demonstrating variety of cells. (*a*) Multinucleated cell, (*b*) fusiform cell with mitosis and thickened process, (*c*) cell "buds." Unfixed, unstained. × 300.



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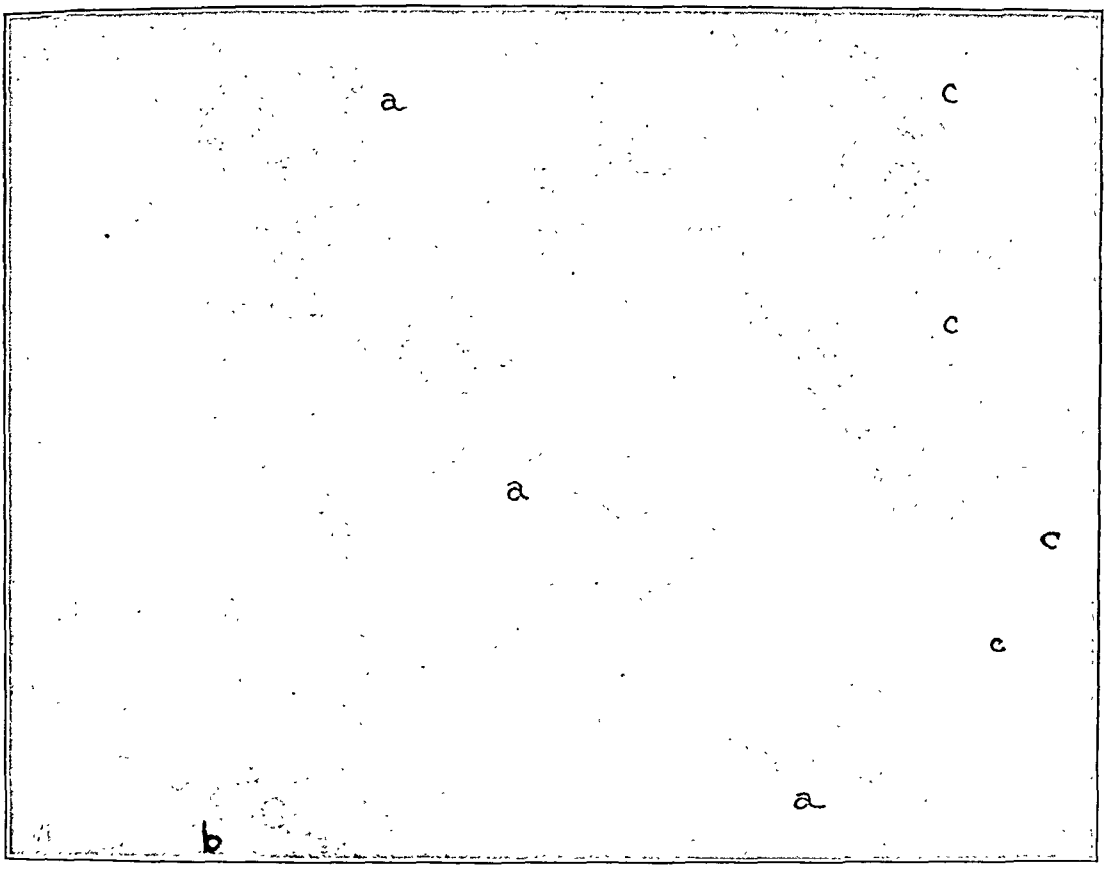


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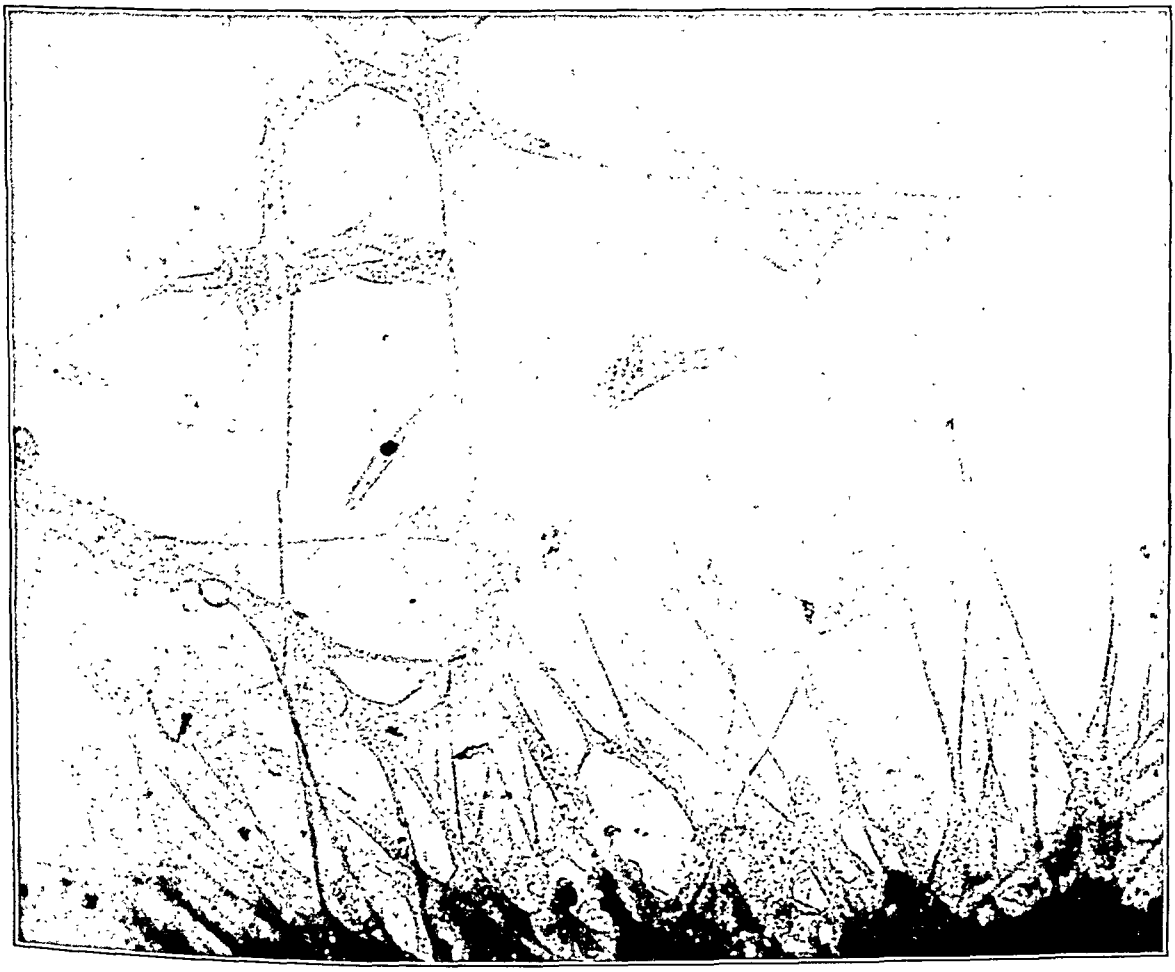
Plate 97

Fig. 3. S 29667. Outer field of growing cells of culture of glioblastoma multiforme showing at (*a*) apparent division at end of mitosis of spindle-shaped cell, at (*b*) multinucleated cell and cell "buds" at (*c*). Unfixed, unstained. × 300.

Fig. 4. S 30556. Edge of explant of culture of glioblastoma multiforme showing multinucleated cells, mitotic figures, cell buds and cells resembling astrocytes. Gold chloride sublimate. × 225.



3



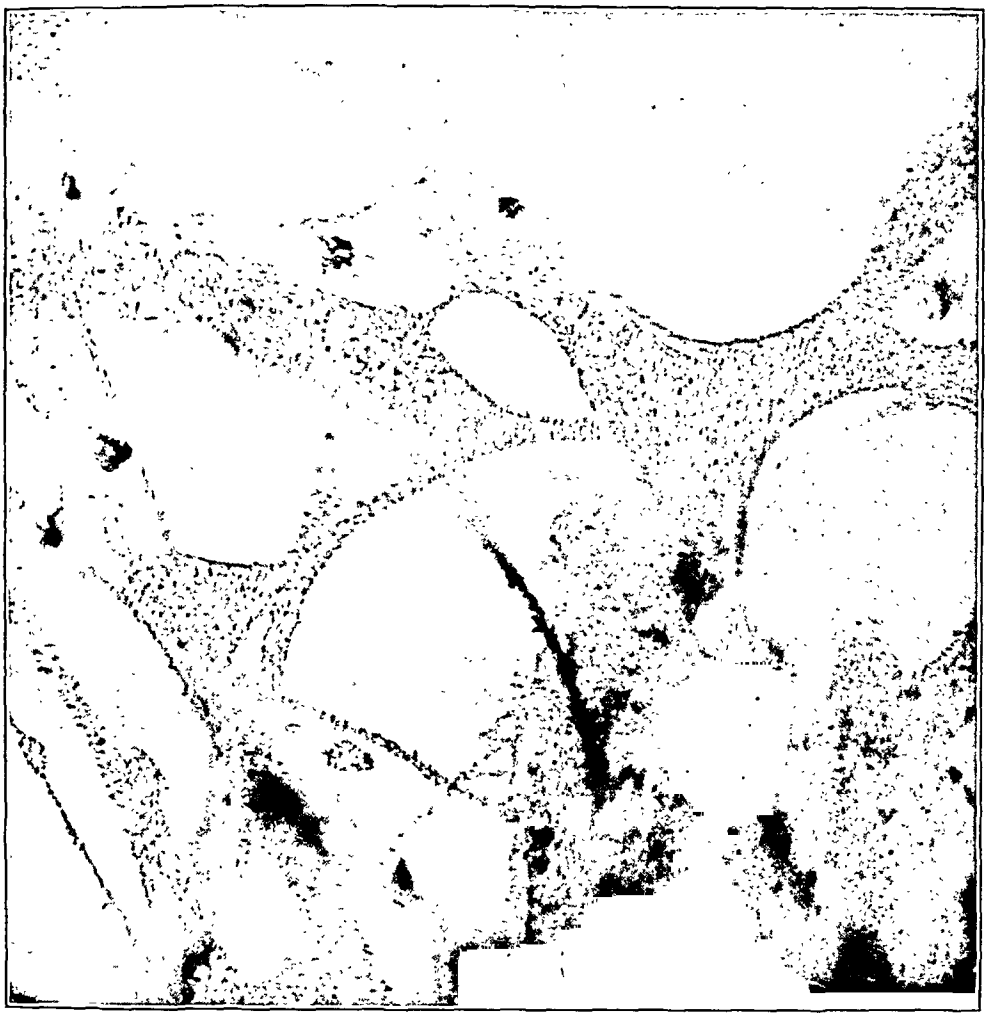
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Plate 98

Fig. 5. S 30556. Mitotic figures in large cells of a culture of glioblastoma multiforme. Gold chloride sublimate. × 850.

Fig. 6. S 30556. Cell of spongioblast type at edge of explant of culture of glioblastoma multiforme. Gold chloride sublimate. × 850.

Fig. 7. S 29667. Large cell bud and irregular mitotic figures in lower cell of a culture of glioblastoma multiforme. Gold chloride sublimate. × 850.



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Buckley

Glioblastoma Multiforme

THE PRODUCTION OF ALLERGIC INFLAMMATION IN THE KIDNEYS *

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INTRODUCTION

Since Arthus [1] first described the phenomenon in 1903, much work has been done upon the local reaction which occurs in the subcutaneous tissues after repeated injections of a foreign protein. Little notice, however, has been given to the occurrence of a similar phenomenon in internal organs. Friedberger [2] produced a cellular pneumonia in sensitized guinea pigs by spraying horse serum into the trachea. Ishioka [3] has reported and confirmed these experiments. Schlecht and Schwenker,[4] after careful histological study of the lungs in this condition, described the proliferation of the cells lining the alveolar walls, and the exudation of eosinophilic leucocytes into the alveoli with the formation of fibrin which gives rise to a type of bronchopneumonia. Opie [5] observed a localized consolidation surrounding a central focus of necrosis with leucocytic infiltration and edema after injecting 0.2 cc. of horse serum through the thoracic wall into the lung of a sensitized rabbit. Shapiro and Ivy [6] produced ulcers of the stomach by injecting foreign protein into the gastric mucosa of sensitized rabbits and dogs. Long and Seyfarth [7] found only a moderate and transient interstitial infiltration of wandering cells after the introduction of egg albumin and gliadin into the testicle of guinea pigs sensitized to these proteins. The mild mononuclear infiltration which resulted did not definitely exceed the similar response to intratesticular injection of these same proteins into normal guinea pigs. As far as we know there has been no work done on anaphylactic inflammation in the kidneys of sensitized animals. Long and Finner [8] attribute a coagulation necrosis of the glomeruli and tubules found in one kidney of a tuberculous pig four days after perfusion with tuberculin protein to anaphylactic inflammation. Longcope [9] and Boughton [10] have reported an experimental nephritis in sensitized animals by repeated proteid intoxication. This, however, was produced by anaphylactic shock and not as a local reaction of the renal tissue.

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Arthus [1] injected rabbits subcutaneously with a foreign protein, every six days and found that local toxicity of the protein steadily increased. The material of the first three injections was absorbed within a few hours; the fourth produced a soft infiltration of the skin which remained two to three days; the fifth a hard infiltration which remained five or six days; the sixth an extensive damage to the subcutaneous tissue which was transformed into a compact, white aseptic mass which remained unchanged for weeks; the seventh a superficial necrosis which terminated in a cutaneous ulcer that healed only after several weeks. The onset of this phenomenon is not dependent upon the number of injections entirely, as there are individual variations and a greater number of injections may be necessary. The reaction is not the result of the amount of protein injected, as only 0.5 cc. of the solution gave a strong reaction, but only in specifically sensitized animals. The preliminary sensitization could be made by any parenteral route. The severity of the local reaction differs with the location of the tissue injected. The reaction occurs most markedly in the subcutaneous tissues of the chest and abdominal wall, while only an edema occurs on the ear.

Arthus and Breton [11] studied this subcutaneous reaction microscopically: Twenty-four hours after injection of the homologous protein into a sensitized rabbit there was edema with many leucocytes, and the connective tissue fibers were doubled in volume. After forty-eight hours this condition was more extensive with hemorrhage in the deeper layers of the skin down to the muscle; this remained stationary for about two days, then the skin sloughed off and there resulted an ulcer, the floor of which was covered with a soft, cheesy, sterile mass composed of disintegrated leucocytes. Gerlach [12] described an earlier stage: Within the first hour there develops a rapidly spreading edema; after an hour there is a marked swelling of the connective tissue fibers with compression of the capillaries causing an anemic necrosis in the central portion; surrounding this area there is edema with precipitation of fibrin, hyperemia, and later stasis in the vessels which became plugged with white blood corpuscles; there is also a massive emigration of leucocytes. Anaphylactic inflammation is characterized by its rapid course and the intensity of the reaction. Rössle [13] speaks of this augmented reaction in the tissues as "the highest inflammation-capacity of the organism."

Opie [14] thinks the above necrosis and inflammation due to the oc-

clusion of the capillaries and small vessels by hyaline thrombi, formed by the reaction of the antigen with its antibody, the leucocyte infiltration being caused by the positive chemotaxis of the precipitate formed. Metalnikow [15] showed that in sensitized animals the antigen possessed positive chemotactic activity. Coca [16] has suggested that the necrosis might be caused by muscular contraction of the arteries. Klinge [17] demonstrated that it was not due to a sensitized nervous system.

Rössle,[18] by using an antigen that could be recognized in microscopic sections, *viz.*, chicken blood, found that it was absorbed much more slowly and with greater difficulty in sensitized than in normal rabbits. The more frequent the injections, the more difficult the resorption; the injected cells at the site of the injection disintegrated locally. In normal animals ordinary leucocytes predominated in the resorptive inflammation, while in anaphylactic inflammation the eosinophiles early predominated.

Auer [19] found that it was not necessary to inject the specific protein locally to produce inflammation. Xylol applied to the ears of a sensitized rabbit which was reinjected intraperitoneally with the antigen, produced a local gangrene by concentrating the antigen in the ears. "A subminimal concentration of antigen for non-inflamed sensitized cells may thus become effective when inflamed sensitized cells are concerned." He suggested that this may theoretically occur in any tissue of a sensitized animal which can show an anaphylactic reaction and that these conditions might explain a number of functional abnormalities in the human subject. Tannenberg,[20] however, using Auer's method, obtained a marked diminution of the inflammatory changes after applying cantharadin to the backs of sensitized rabbits.

Nicolle [21] demonstrated in 1907 that this local inflammation was of an anaphylactic nature in that it occurred in animals that were passively sensitized. It is, therefore, dependent upon specific substances transmissible in the serum.

Opie [5,22] has done extensive work on anaphylactic inflammation in the light of modern conceptions of anaphylaxis. He contributed the following: (1) the reaction depends on the simultaneous presence of antigen and antibody in the tissues; (2) the severity of the reaction varies with, but does not exactly parallel, the precipitin titer of the serum; (3) the reaction varies in different species; (4) the local reac-

tion protects the animal from a general reaction by limiting the antigen to the site of injection; (5) a total desensitization of the tissues and disappearance of the precipitin follows the response to a pure antigen such as crystalline egg albumin, but not to a complex one as egg-white or horse serum.

Grineff [23] described a therapy for anaphylactic inflammation. He found that intravenous injections of small quantities of a foreign protein prevented the subcutaneous reaction to the local injection on the following day.

Wells [24] and Opie [14] have suggested, as has Auer, [19] that anaphylactic inflammation might be produced in other tissues than the skin under similar conditions.

Method

The proteins used in these experiments were commercial horse serum (Parke, Davis and Co.) and a 2 per cent solution of crystalline egg albumin (Merck) made fresh each time in Ringer's solution. Rabbits were used for all the experiments and were sensitized by the original method of Arthus. The subcutaneous injections of 1 cc. of horse serum or 2 cc. of egg albumin were made into the abdominal wall. The injections were every five or six days, alternating the side each time. They were continued until extensive necrosis of the skin was obtained which assumed a purplish black color. The next injection, five days later, was made directly into the left kidney. To do this an assistant holds the rabbit in a flexed position; the kidney is grasped through the abdominal wall and is brought up and held firmly immediately under the skin. With a small hypodermic needle and under aseptic conditions, 0.5 cc. of the protein is injected directly into the kidney. The feel of the needle as it passes through the renal capsule indicates that the injection is being made into the substance of the kidney. The kidneys were injected from one to eight times at five or six day intervals. The rabbits receiving from one to four injections in the kidney were killed twenty-four and forty-eight hours after the last injection. Four groups of rabbits received five six, seven, and eight injections respectively, in the kidney, and animals from each group were killed at intervals of forty-eight and seventy-two hours, one, two, and three weeks and one month. The right kidney of each rabbit was used as a control, except in eight cases. In these rabbits the left kidney was injected three times and at

the same time as the last injection, the right kidney was injected and the animals killed in twenty-four and forty-eight hours. At autopsy, samples of urine were obtained from the bladder with which albumin tests and microscopic examinations were made.

At the time of the first injection in the kidney, blood was taken from the ear for precipitin tests. The serum was run to progressive dilutions of antigen by the ring method, the tubes standing in a water bath at 37° C for one-half hour.

Control rabbits received one to eight intrarenal injections and were killed twenty-four and forty-eight hours after the last injection in the left kidney. Seven were injected with 0.5 cc. of a sterile 2 per cent solution of Silver Label gelatin in Ringer's solution. Eight were injected with egg albumin without being previously sensitized. One was injected with India ink in both kidneys and killed after the right had been injected thirty minutes and the left one hour.

Results

The average number of injections of horse serum required to produce necrosis of the skin was seven; of egg albumin, six. Eight rabbits having from thirteen to twenty-one subcutaneous injections showed a very slight reaction. This confirms Arthus' work that all animals do not react to the same degree. Four rabbits that had been sensitized to horse serum one year before showed a marked skin necrosis after the second injection. The horse serum and the egg albumin produced the same type of reactions in the kidneys.

The results of the precipitin titrations agreed with those of Opie's mentioned above, namely, that the severity of the reaction varies with, but does not exactly parallel, the precipitin titer of the serum.

Of fifty-four sensitized rabbits, thirty-two were killed within seventy-two hours after the last injection in the left kidney, and twenty-two after seventy-two hours. Twenty of the thirty-two rabbits killed within seventy-two hours showed hemorrhage associated with necrosis at the site of injection in the left kidney, seven in the right kidney. One killed in four days showed a small hemorrhage. The fact that the red cells were all well preserved was evidence that the hemorrhage was due to the last injection. Six not included in the twenty showed hemorrhage beneath the renal capsule only. One of the right kidneys injected had a hemorrhage beneath the capsule only. Six

showed no evidence microscopically of having been injected in the kidney.

TABLE I

Hemorrhage and Necrosis in Sensitized Rabbits

Number of rabbits	Left kidney		Right kidney
	Autopsy within 72 hrs.	Autopsy after 72 hrs.	Autopsy within 48 hrs.
Sensitized	32	22	8
Showing hemorrhage and necrosis	20	1	7
In pyramid and medulla	14	1	2
In cortex	6	0	5
With hemorrhage underneath capsule	6	0	1
Not showing hemorrhage	6	21	0
With recent scar tissue	8	22	0

No recent hemorrhage was observed in the rabbits killed one or more weeks after the last injection. The injected kidneys of all of these had some recent scar tissue, or hemosiderin was present in necrotic areas undergoing organization. Eight that were killed within seventy-two hours and showed recent scar tissue had had at least three injections in the kidney, the scar tissue resulting from an earlier injection.

When the hemorrhage occurred in the medulla or pyramid, the amount of necrosis exceeded that of hemorrhage. The areas of hemorrhage and necrosis in this part of the kidney had a very definite outline. That the necrosis was due chiefly to the reaction of the sensitized tissues to the antigen is shown by the absence of necrosis around the hemorrhages in the control rabbits. The definite outline of the necrosis may have been related to the less highly specialized tissue of the medulla and pyramid; or possibly, to peculiarities of the histologic architecture of this portion of the kidney.

The hemorrhage and necrosis was greatest when the protein was injected into the cortex and the animal killed in twenty-four hours. This necrosis did not surround the hemorrhage evenly but spread out irregularly from all sides, but most markedly toward the capsule. That the material injected did not diffuse out in the surrounding tissue to any great extent was demonstrated in the kidneys injected with India ink. The kidneys with areas of necrosis had had from one

to three injections, except one kidney which had received seven injections. Those that showed a large amount of necrosis without hemorrhage in the cortex had had five injections, the necrosis being the result of a previous injection, as there was a definite increase in the connective tissue.

In the necrotic areas of the cortex, the epithelium of all types of tubules was swollen and showed necrosis. Few of the glomeruli were necrotic, most of those in and adjacent to the area of necrosis were distended with blood. The connective tissue was affected least, sometimes only showing edema in an area of necrosis, and soon started to proliferate. There was a variable infiltration of leucocytes in and around the area of necrosis; only a few were eosinophiles. Hyaline and blood casts were found in most of the collecting tubules near this area. In all the kidneys except two, the necrosis was of the coagulation type. In these two there was liquefaction necrosis due to an extensive infiltration of polymorphonuclear leucocytes.

The following protocols illustrate the results:

RABBIT NO. 2

Feb. 1 to Apr. 19: Received twenty-one subcutaneous injections of horse serum in the abdominal wall, had a two plus skin reaction,* and a precipitin titer of 40,000.

Apr. 23: Injected 0.5 cc. of horse serum into the left kidney.

Apr. 27: Injected 0.5 cc. of horse serum into the left kidney.

Apr. 28: Autopsy.

Urine: None obtained.

Description of Left Kidney; Gross: Two needle punctures were found, one containing a blood clot which on the cut surface extended into the substance of the kidney for about 7 mm.

Microscopic: A large hemorrhage extends from the cortex to the pyramid. In an area at one side of the hemorrhage extending from the medulla up to the cortex, there is marked necrosis of the tubules and a slight edema. A few of the glomeruli nearest the hemorrhage are necrotic, and a few are distended with blood. There is only a slight infiltration of leucocytes in the necrotic area, but a larger number surround the hemorrhage. There are a great many hyaline casts in the collecting tubules in and near the area of necrosis.

* One plus represents a slight edema for twenty-four hours; two plus, greater edema lasting for five days, then disappearing; three plus, hard fibrotic induration lasting a much longer time; four plus, necrosis with ulceration.

Description of Right Kidney: Grossly and microscopically normal.

RABBIT NO. 101

Dec. 17 to Jan. 13: Received five subcutaneous injections of egg albumin in the abdominal wall, had a four plus skin reaction, and a precipitin titer of 5000.

Jan. 17: Injected 0.5 cc. of egg albumin into the left kidney.

Jan. 21: Injected 0.5 cc. of egg albumin into the left kidney.

Jan. 25: Injected 0.5 cc. of egg albumin into the left kidney and 0.5 cc. into the right kidney.

Jan. 26: Autopsy.

Urine: albumin, positive; microscopic, few hyaline and many granular casts, few white blood cells.

Description of Left Kidney; Gross: The upper pole had a small hemorrhage underneath the capsule and a pale area on the surface of the kidney at the site of an injection. The lower pole was larger than normal and hard. A large pale area covered the central and lower parts of the kidney. The cut surface appeared normal.

Microscopic: There is a slight hemorrhage along the needle puncture which terminates in a larger one in the medulla and pyramid. In the lower portion of the cortex at the beginning of the large hemorrhage there is necrosis of the tubules and a marked infiltration of leucocytes. There is also a marked infiltration of leucocytes at the lower edge of the hemorrhage. An occasional hyaline and blood cast was seen in the collecting tubules near the necrosis and many below the hemorrhage.

Description of Right Kidney; Gross: A small white area was found on the surface at the site of the injection; the whole kidney was paler than normal. The cut surface looked normal.

Microscopic: Above and to one side of a small hemorrhage in the lower part of the cortex, the tubules are necrotic and all the glomeruli in this area are affected. There is a slight edema and leucocytic infiltration above the hemorrhage with a greater infiltration of leucocytes in the more marked area of necrosis near the surface of the kidney.

RABBIT NO. 106

Dec. 17 to Jan. 13: Received five subcutaneous injections of albumin in the abdominal wall, had a four plus skin reaction, and a precipitin titer of 5,000.

Jan. 17: Injected 0.5 cc. of egg albumin into the left kidney.

Jan. 21: Injected 0.5 cc. of egg albumin into the left kidney.

Jan. 25: Injected 0.5 cc. of egg albumin into the left kidney, and 0.5 cc. into the right kidney.

Jan. 26: Autopsy.

Urine: albumin, strong positive; microscopic, few granular casts and many white blood cells.

Description of Left Kidney; Gross: Three needle punctures could be seen; the capsule was adherent over each one. The lower pole was hard, but not as hard as in Rabbit No. 101. The cut surface showed a hemorrhage along one needle puncture.

Microscopic: There is a slight hemorrhage near the surface of the kidney with necrosis of all the tubules surrounding this area and a moderate infiltration of leucocytes and fluid between them. The glomeruli around this area are distended with blood, two have been replaced by a mass of leucocytes.

Description of Right Kidney; Gross: The cut surface showed a hemorrhage in the pyramid. Otherwise the kidney was normal in appearance.

Microscopic: A long narrow hemorrhage (along the needle puncture) appears in the medulla. There is a very slight necrosis of the tubules surrounding the hemorrhage with a moderate infiltration of leucocytes.

Discussion

The inflammatory reaction in the sensitized animals was marked, whereas in the control rabbits there was only hemorrhage due to mechanical injury. The anaphylactic inflammation produced in the kidneys of these animals differed from other forms of inflammation. (1) In the rate of progress. In twenty-four hours a far advanced acute inflammatory reaction was present. Examinations at earlier stages have not been made. (2) In the relatively slight edema in the inflammatory areas in these kidneys as compared with similar areas in the subcutaneous tissues. This was probably due to the small amount of connective tissue in the kidney and to the effect of the dense capsule upon the increase of intrarenal pressure by relatively small exudates. The exudate appeared to consist chiefly of leucocytes with relatively little fluid. (3) In the extent of the necrosis which is primarily of the coagulation type. Liquefaction occurred, as in other forms of inflammation, only after infiltration of the area with polymorphonuclear leucocytes.

The cause of the necrosis is not entirely clear. Three possibilities suggest themselves. (1) Infarction due to thrombosis. Although thrombi were occasionally found, they did not appear to be numerous enough to account for the necrosis. (2) Gerlach believed that in areas of anaphylactic inflammation of the skin edematous swelling

of the connective tissue reached such a degree that compression of the capillaries and venules caused a prolonged ischemia followed by necrosis. The edema in the kidneys from these experiments does not appear to be sufficient to account for the necrosis. (3) The probable cause of the necrosis was the direct toxic effect of the antigen on the sensitized tissues. This is suggested by the fact that the more highly specialized and differentiated tubular epithelium of the cortex appeared to be more susceptible to injury than the less highly specialized epithelium of the collecting tubules of the pyramids. Opie [25] has shown that in sensitized animals the antigen remained localized in the tissues instead of being quickly reabsorbed as in non-sensitized animals. The antigen remaining concentrated at the site of the injection caused the necrosis and inflammation to be localized and more intense.

Conclusions

1. Hemorrhage was found in all the injected kidneys when the animal was killed within seventy-two hours.

2. Necrosis was observed in kidneys of sensitized rabbits only.

3. Anaphylactic inflammation differs from other inflammation in the intensity and rapidity with which it occurs.

4. This anaphylactic inflammation was caused by the direct toxic effect of the antigen on the sensitized tissues.

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THE EFFECT OF COMBINED POTASSIUM IODIDE AND THYROID SUBSTANCE UPON THE THYROID GLAND *

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In studies made of the effect of potassium iodide and thyroid substance upon the normal thyroid gland of the guinea pig it has been shown [1, 2] that KI has a marked stimulating effect which is most pronounced after from 15 to 20 daily doses of 0.1, 0.05, or 0.01 gm., whereas thyroid substance has a definite depressing effect. In this paper we wish to report our findings concerning the effects of feeding thyroid substance alone, the effects of feeding KI and thyroid together to the same guinea pig and also the effects of feeding one of these substances following a course of the other substance. In a few animals some of the previous experiments were repeated so as to have a control check.

The method used has been described previously [1, 2] and need not be mentioned again. The experiments may be grouped as follows: (1) Two untreated guinea pigs; (2) 2 guinea pigs fed with 0.1 gm. KI daily for twenty days; (3) 11 guinea pigs fed with 0.1 gr. of thyroid substance (Armour & Co.) daily, 6 for thirty days, 5 for twenty days; (4) 14 guinea pigs fed with both KI and thyroid substance, 6 for twenty days, 4 for thirty-two days, 4 for thirty-eight days; (5) 4 guinea pigs fed with KI for twenty days and then thyroid substance for twenty-three days; (6) 3 guinea pigs fed with thyroid substance for thirty days followed by KI for twenty days.

Untreated Control Guinea Pigs: The number of mitoses was 348 and 195. This is well within the control figures.[1, 2] The acini were medium-sized, the epithelium medium cuboidal, the colloid hard with occasional vacuolization and few phagocytes.

KI-Fed Guinea Pigs: The mitoses were very numerous, 2520, and 4364. The epithelium was relatively tall, cuboidal and columnar; the colloid was soft and phagocytes were numerous.

Thyroid Substance-Fed Guinea Pigs: In the description by Gray and Loeb [1] of the effect of thyroid substance upon the thyroid gland

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of the guinea pig, they used only four animals in which accurate quantitative estimation of the mitotic activity was made. These experiments were repeated upon a large series. Since the same results were obtained from both the twenty- and thirty-day feedings with thyroid substance, we shall not differentiate between these two groups in our study.

TABLE I

Number of animals	Number of mitoses	Number of animals	Number of mitoses
5	0	1	20
1	16	2	80
1	18	1	166

Average 34

Table I enumerates the number of mitoses found in the entire thyroid gland; it varies between 0 and 166, with an average of 34. This number is somewhat lower than the average of 64 that Gray and Loeb found; it is markedly lower than the average for the normal control.

Structurally, the thyroid gland showed slight but definite changes. The acini were small and medium in size, being within the range of the control, although on the whole, one would say that the acini were smaller than those found in the thyroid glands of the untreated animals. This difference is too slight to deserve much emphasis. The epithelium was a little lower and the colloid a little harder with less vascuolization than was usually seen in control animals. These experiments show therefore that the feeding of thyroid extract to guinea pigs for a period of twenty to thirty days produces the structural signs of inhibition in the activity of the gland.

The Effect of a Simultaneous Feeding of KI and Thyroid Substance: In this series of experiments 0.1 gm. KI and 0.1 gr. thyroid substance were given daily to the same animal. Of these animals 6 were thus fed for twenty days, 4 for thirty-two days and 4 for thirty-eight days. The number of mitoses obtained in each case is listed in Table II.

It will be noted from Table II that after a period of twenty days of a combined thyroid and KI feeding, the number of mitoses in the entire thyroid gland is very much the same as that found in the un-

TABLE II

20 days	32 days	38 days	20 days	32 days	38 days
398	96	0	18	16	28
188	240	0	0	—	—
0	0	0	570	—	—
	Average	196	88	7	

treated control. If the combined feeding is continued, however, for periods of thirty-two and thirty-eight days, there results a marked diminution in the number of mitoses.

After twenty days of combined thyroid and KI feeding the epithelium becomes somewhat taller than the average untreated control. It is definitely taller than that observed in the glands of animals that have been fed exclusively with thyroid and lower than that seen in animals fed with KI alone. The size of the epithelium under these conditions is somewhat midway between that found in the glands of animals fed with thyroid and those fed with KI. The colloid, on the other hand, is very hard and poorly infiltrated with phagocytes; it is very similar to that seen in animals fed with thyroid substance.

Guinea Pigs Fed with KI for 20 Days Followed by Thyroid Substance for 23 Days: In this series 4 guinea pigs were fed first with a daily dose of 0.1 gm. KI for a period of twenty days and then fed 0.1 gr. thyroid substance for a period of twenty-three days. The number of mitoses per gland in individual cases varied as follows: 54, 48, 32 and 0, giving an average of 33 mitoses. This figure is similar to that obtained in the control series that were fed with thyroid (see above). It appears, therefore, that a preliminary administration of KI to the animal does not counteract in any way the subsequent depressing effects of thyroid substance on the mitotic cell activity of the acinar epithelium. In accordance with this view, we also find the microscopic appearance of the gland to resemble very closely the gland of the control thyroid-fed animal in so far as the colloid is concerned. It is very hard and free from phagocytes; the epithelium, however, is somewhat taller than that seen in animals fed with thyroid alone.

Feeding of KI Following a Preliminary Administration of Thyroid Substance: In these experiments 3 guinea pigs were first fed with daily doses of 0.1 gr. thyroid substance for thirty days, followed by

0.1 gm. potassium iodide for twenty days. In these animals the number of mitoses was 2296, 788 and 656, respectively, giving an average number of 1246 mitoses per entire gland. This figure, although considerably lower than that found in the series that were fed with KI alone, is nevertheless much greater than the average number in the normal controls (see above). Furthermore, the microscopic appearance of these glands is very similar to that noted in the control KI-fed animals; the colloid is softened and very rich in phagocytes, while the acinar epithelium is proportionately increased in size. It follows therefore that a previous administration of thyroid substance to the animals hinders only very little the subsequent effects of KI in so far as structural or proliferative changes in the gland are concerned.

Discussion

The results obtained in these experiments confirm the previous findings as to the manner of the action of potassium iodide and thyroid substance on the activity of the thyroid gland of the guinea pig.

Table III

Normal control	KI feeding control	Thyroid feeding control	KI plus thyroid 20 days	KI plus thyroid 32 days	KI plus thyroid 38 days	KI 20 days thyroid 23 days	Thyroid 30 days KI 20 days
271	3442	34	196	88	7	33	1246

Table III summarizes the average number of mitoses found in the thyroid gland under the different experimental conditions. It shows that KI feeding to normal guinea pigs causes increased cell division, whereas thyroid feeding produces exactly the opposite effect, namely a diminution in cell proliferation. With the combined feeding there was no balance between these two substances, for in twenty days the number of mitoses is not midway between the figures obtained for the KI animals and the thyroid animals, but is very nearly that obtained with thyroid feeding; yet there may be a slight neutralizing effect noticeable. In the thirty and thirty-eight days the thyroid effect is complete; however, in this connection we must recall the fact that a diminished number of mitoses has been found

in KI feeding after twenty-five to thirty days. The other structural changes noted in the gland are essentially those of thyroid feeding.

There are definite average differences in the size of the epithelium between the normal controls, the KI-fed and the thyroid-fed guinea pigs. In the animals which received combined feeding of KI and thyroid, the epithelium is a little taller than in the average control; although the gland in this case is principally under the influence of the thyroid substance, still there is some evidence of KI stimulation. A similar sign of KI stimulation is noted in the fact that in the gland of the animals fed with thyroid for twenty-three days following a preliminary twenty days KI feeding, the epithelium is taller than found in twenty-day thyroid feeding. The picture here is similar to what we have noted when feeding small doses of KI to normal guinea pigs.[3]

The colloid in the animals which received combined feeding, even in the early period of twenty days, is hard and resembles the colloid in the controls fed with thyroid. From the numerous experiments that have been carried out in our laboratory, it is certain that KI has a stimulating effect upon the epithelium of the thyroid gland which is still noticeable when this substance is antagonized by administration of thyroid substance. As far as the colloid is concerned KI and thyroid substance also act antagonistically, the former substance tending to soften it through a direct action of the phagocytes, which are increased in number, upon the colloid. Thyroid substance, on the other hand, causes a hardening of the colloid as well as a diminution in the number of phagocytes.

In the glands of animals fed with KI following thyroid substance the character of the epithelium and of the colloid is similar to that observed in the glands of animals fed with KI that have not received a preliminary treatment with thyroid substance, the number of mitoses seems, however, to be somewhat diminished in these cases. We find thus that the interaction between thyroid substance and KI is similar to the interaction between KI and anterior pituitary substance (Armour & Co.) when fed by mouth, which McCordock[4] has recently described. McCordock finds that through combined feeding of anterior pituitary substance and KI the effect of the latter can be approximately neutralized, although he could also find signs of the effects of the individual substances upon different parts of the gland.

Summary

1. Feeding of potassium iodide to normal guinea pigs stimulates the thyroid gland, whereas feeding of thyroid substance produces the opposite effect.

2. In feeding KI and thyroid concomitantly, the effect of KI is evidenced by the increased size of the epithelium, and the effect of thyroid substance is manifested by the hardness of the colloid and the diminished number of mitoses.

3. Preliminary feeding of KI interferes only slightly with the subsequent depressive action of thyroid substance and similarly a preliminary feeding of thyroid substance affects only to a small extent the subsequent stimulative effects produced by KI feeding.

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PATHOLOGIC HISTOLOGY OF ADRENALECTOMIZED CATS*

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The correlation of the changes occurring during Addison's disease with those of experimental adrenal insufficiency has progressed slowly. In most of the experimental work the animals have survived for too brief a period for easily recognizable pathologic changes to occur. Conversely, in human cases, adrenal insufficiency has been complicated by other factors. Complications may also occur in experimental adrenal insufficiency as noted in this paper and also by Zwemer and Lyons.[1] As shown by an analysis of our cases of uncomplicated adrenal insufficiency, the histologic changes are roughly proportional to the length of survival after adrenal removal, and were first distinguished in our longest surviving animals.

The experimental work included the gross and microscopic examination of the tissues obtained from ten cats that had had both adrenals removed. The survival period following adrenalectomy averaged 240 hours, and varied between about 100 and 300 hours. No effort was made to prolong the life of these animals by administration of solutions or extracts. However, we exercised particular preoperative and postoperative care and strict asepsis during operations. As controls we used five normal, healthy cats, two cats that were kept continually in deep anesthesia with ether for ten successive hours, two cats that were starved, and two cats that died from bilateral confluent pneumonia.

To obtain fresh tissues, the animals were killed when they appeared extremely feeble. Complete autopsies were performed including the brain and upper portion of the spinal cord. Tissue was cut in small pieces 2 to 3 mm. in thickness, fixed in Zenker's solution,

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formalin, and alcohol-formalin, embedded in celloidin and paraffin, and stained with hematoxylin and eosin, and eosin and methylene blue. Frozen sections of formalin-fixed tissue were stained with Sudan IV, Nile blue sulfate, and osmic acid.

Cultures were taken at the time of the autopsy from the peritoneal cavity and heart's blood and planted in plain broth, and milk, and also on sheep blood agar plates. In this series of ten test animals we have included only those in which the cultures were sterile. Nearly as many animals in which we obtained positive cultures had to be discarded. The survival period of the infected animals was much shorter than that of the uninfected group. This agrees with the known susceptibility of adrenalectomized animals to toxins.

A gross examination of the organs and tissues of the animals suffering from adrenal insufficiency reveals little that is unusual. The amount of subcutaneous fat is diminished, although the fat in the mesentery and great omentum is still quite abundant. The body is dehydrated, the surfaces of the peritoneal cavity though smooth, lack their usual sheen. The liver and spleen are moderately congested, and the blood that flows from the freshly cut surfaces is unusually viscid. The subcutaneous, mesenteric and mediastinal lymph nodes are enlarged, firm and pale pink. The kidneys are large, the capsules strip readily from opaque yellow smooth surfaces. The fresh surface of the kidney is rather startling, the cortex is so opaque, yellow, regular and thickened, in contrast to the pyramids which are moderately congested. The heart, lungs, pancreas, intestines, bladder, skeletal muscle, testis, brain, cord, pituitary, thyroid, parathyroid, aorta and bone marrow show no constant gross lesions.

In résumé, the gross findings are fatty degeneration of the kidney, enlargement of the lymph nodes, dehydration, and moderate congestion of the liver and spleen.

MICROSCOPIC REPORT

The histologic changes which appear to be constantly associated with adrenal insufficiency are confined to the kidney, lymphoid tissue throughout the body, thyroid and testis. The lesions were alike in all cases and varied only in degree, depending largely on the period of survival.

Kidney: The microscopic findings in the kidney are even more

striking than the gross picture. The lesion is confined to the tubules, especially the convoluted portions, leaving the glomerular capsule and capillary tufts unaffected.

The tubular epithelium from the kidney of a normal cat contains a moderate amount of fat which varies slightly in different animals. In the adrenalectomized cat the amount of fat in the epithelial cells lining the tubules is greatly increased.

In comparing the kidneys from the normal cats, the cats that had received a large dose of ether, those that died from an acute infection, and the adrenalectomized cats, the contrast between the last named group and the first three is quite remarkable. On the other hand there was no appreciable variation in either the appearance, staining reaction or amount of visible fat in the tubular epithelium of the normal, starved, and etherized cats.

The normal kidney, in the eosin-methylene blue preparations, has convoluted tubules that have very distinct lumina bordered with well defined, rather cuboidal epithelium. The cytoplasm of these cells, which is fairly dense, homogeneous and eosin-staining, contains minute to medium-sized clear vacuoles that are usually situated toward the base of the cell. With the common differential fat stains these vacuoles are shown to represent fat droplets that stain orange with Sudan IV, black with osmic acid and pink with Nile blue sulfate. No visible fat was present in the glomeruli, in the lumina of the tubules, in the epithelium of the collecting tubules, in the stroma or in the blood vessels.

In the kidney of the adrenalectomized cat the cells lining the convoluted tubules are poorly defined, irregular and so distended with large and small vacuoles that they appear to obliterate the lumina. Even the cytoplasm of the cells lining the loops of Henle and the collecting tubules contains large and small clear vacuoles. Sections stained with Sudan IV, Nile blue sulfate, and osmic acid show no fat in the glomeruli. The fat in the convoluted tubules is very abundant and stains orange-red with Sudan IV, black with osmic acid and pink with Nile blue sulfate. In the collecting tubules the fat stains quite differently; with Sudan IV, it appears as fine orange-yellow granules, which fail to stain with osmic acid, and stain dark blue with the Nile blue sulfate. Furthermore, small fatty casts are seen in the lumina of the tubules. These stain orange-red with Sudan IV, and black with osmic acid, whereas with Nile blue sulfate the casts in

the convoluted tubules stain pink and those in the larger collecting tubules stain both pink and blue. This differentiation suggests that the source of the casts is probably in the tubular epithelium; the pink-staining casts arising from degenerating cells of the convoluted tubules and the blue from the more distal collecting tubules.

Small fat droplets were frequently seen in the larger blood vessels. These stained orange with Sudan IV, black with osmic acid, and some blue and some pink with Nile blue sulfate.

In summing up the histologic changes in the kidney, it is clear that the tubules show a definite lesion in which the amount of fat is greatly increased. In the convoluted tubules this stains as neutral fat, while in the collecting tubules it appears as fatty acids. In the collecting tubules fatty casts are seen that probably arise from the lipoid degeneration of the tubular epithelium.

Lymphoid Tissue: In both the spleen and lymph nodes there is marked lymphoid hyperplasia. The lymph nodules are increased in size, the germinal centers are large and filled with young lymphocytes and lymphoblasts in which mitoses are numerous. Mature lymphocytes distend the sinuses and infiltrate the stroma of the lymph nodes.

This finding is entirely different from the observation of Banting and Gairns [2] on dogs, in which the lymph nodes showed enlargement with edema, a tremendous proliferation of plasma cells, endothelial leucocytes, enlarged macrocytes and occasionally polymorphonuclear leucocytes, and also a great increase in the reticular tissue, compression and almost complete obliteration of the germinal centers, active phagocytosis of red and white blood cells, and frequently blood pigment.

Zwemer and Lyons have recently observed that there is an absolute increase in the lymphocytes in the circulating blood of adrenalectomized cats, a finding that may be correlated with the histologic changes in the lymphoid tissue in our series of "test" animals.

Thyroid: In most of the animals and especially in those with longer survival periods, the follicles show a moderate degree of colloid distention. The colloid is homogeneous and stains a pale pink with eosin. The follicular epithelium is low cuboidal or slightly flattened.

Testis: The interstitial cells of the testis are increased in size and number. Their cytoplasm is distended with minute clear vacuoles of

uniform size. They morphologically resemble the cells in the outer layer of the cortex of the suprarenal gland. It is said that the number and size of the interstitial cells vary greatly under normal conditions, hence our observation, which is based on a rather small group of animals, may simply be a chance finding. However, this increase occurred in all of the test animals and appeared to vary with the duration of survival. When stained with Sudan IV, osmic acid or Nile blue sulfate the cells resemble in every way those of the cortex of the normal adrenal gland of the cat.

Discussion

Several points must be carefully considered in interpreting the histologic changes that appear to follow bilateral adrenal removal. First, are the changes merely constitutional variations that might be found in any group of animals, or are the changes constant, and do they vary in degree depending on the period of survival? Secondly, can one be certain that removal of the adrenal glands leaves no accessory adrenal tissue, and what part does infection, ether inhalation, dehydration or thirst play in producing the histologic changes?

In answer to the first question we feel that the histologic findings are quite different in the "control" and "test" animals, and cannot be accounted for simply on the basis of unusual constitutional variations within the normal. Moreover, these changes occurred with definite regularity and in most cases were accentuated in the long-surviving animals.

It is impossible to be absolutely certain that all adrenal tissue has been removed, yet in the cat the presence of accessory adrenal tissue is a very unusual finding, and if present even in small amounts will probably maintain the life of the animal. An example from one of our own series will make this point clear. A large cat that made a good recovery after bilateral adrenalectomy, began on the second day after the operation to show signs of adrenal insufficiency which slowly progressed for about eight days. The animal then remained in a lethargic semicomatose state for several days when definite signs of improvement were noticed. The animal gained in weight and showed normal behavior and lived as a normal appearing animal for sixty-seven days. At this time an exploratory operation was done in search of accessory adrenal tissue. A clean dissection was

made of the old adrenal beds and of loose tissue lying adjacent to the large vessels. Multiple sections were taken from this and revealed in addition to scar tissue, lymph nodes and sympathetic ganglia, a small encapsulated nodule of large adrenal cortical cells that were finely vacuolated. The animal lived thirteen days longer showing progressive signs of adrenal insufficiency until death.

Every effort was made during the operations and in the after care of the animals to prevent infection. Cultures, taken from the heart's blood and peritoneum of the animals that we have included in the normal control and operated series, were sterile.

Prolonged ether inhalation, starvation or dehydration, produced no constant lesions that would be confused with the histologic lesions we have described.

Another question that at once suggests itself is what relationship does adrenal insufficiency bear to the histologic lesions? Are these lesions dependent primarily on lack of adrenal tissue and more particularly the cortical portion, or are they secondary to metabolic or biochemical changes resulting from adrenalectomy? On the other hand, may not the chemical changes be simply the result of degenerative changes in the kidney, thyroid and other organs? Such questions as these cannot be answered from a purely histologic study but there are several points that may have a clinical bearing and warrant further consideration.

The problem of "lipoid nephrosis" is one that is receiving a great deal of attention at the present time from both clinicians and pathologists. If we restrict ourselves to the pathologic interpretation of the term whereby we imply simply fatty degeneration of the tubules of the kidney, as was done by Hartman and his collaborators,[3,4] then we may say that adrenalectomy is at least one means of producing this pathologic entity. However, the term "lipoid nephrosis" is in reality a name applied to a clinical syndrome of which a fatty degenerative change in the tubules of the kidney is but one, and probably, a relatively late manifestation.

The characteristics of "lipoid nephrosis" as reported by Epstein [5] are edema, albuminuria, low basal metabolism and changes in the composition of the blood, with absence of hypertension, retinitis, hematuria and azotemia. The changes in the blood are a decrease in the total serum protein, with an inversion of the normal albumin-globulin ratio, and in most cases a decrease in the blood calcium.

No edema is present in animals suffering from adrenal insufficiency. On the contrary, the tissues appear dehydrated (Zwemer [6]). The lowered blood pressure (Marshall and Davis [7]) and fall in basal metabolism (Aub, Forman and Bright, [8] Aub, Bright and Forman, [9] Zwemer and Bright [10]) are similar in both conditions. There are increases in total blood solids (Donath, [11] Hartman et al., [3] Lucas [12]) and in serum calcium (Kisch, [13] Hartman, [3] Stewart and Rogoff [14]) after adrenal removal, in contrast to the "lipoid nephrosis" observations.

Although there are not many points in common with adrenal insufficiency, the changes in blood pressure, basal metabolism, and the kidneys suggest a possible endocrine disturbance present in the clinical syndrome known as "lipoid nephrosis."

Perhaps the most interesting lesion was the marked hyperplasia of the lymphoid tissue. Several years ago the clinical relationship of status lymphaticus and adrenal insufficiency was described (Hedinger,[15] Wiesel,[16] Fahr and Reiche [17]). It would appear that there might be experimental evidence to substantiate that observation, and further it suggests that the lymphoid tissue, whatever its function is, may be physiologically related to the glands of internal secretion. On the other hand the hyperplasia may be only the response to an unusual toxemia. The histologic picture of the lymph nodes substantiates the lymphocytosis previously described by Zwemer and Lyons.

In a few cases of Addison's disease in which the testis was studied microscopically, unusually large and numerous interstitial cells were observed. These and our experimental observations indicate a possible adrenal-interstitial relationship, which is strengthened when we remember their common embryological origin, and the data collected by Glynn [18] on the relation of adrenal hypertrophy to clinical sex anomalies.

It is exceedingly difficult, if not impossible to determine the physiologic activity of a tissue by examination of fixed and stained sections thereof, but, the thyroid glands in our operated animals appear to be in what is frequently called the "resting state." If this is true, it may account in part for the decrease in basal metabolism after adrenalectomy and add something to the interpretation of thyroid-adrenal activity.

SUMMARY

1. The gross and miscroscopic lesions following experimental adrenal insufficiency in cats (average survival ten days) are described.

2. Briefly they consist in fatty degeneration of the tubules of the kidney, lymphoid hyperplasia, moderate colloid distention of the thyroid, and hyperplasia of the interstitial cells of the testis.

3. The significance of these, and other observations on experimental adrenal insufficiency, is discussed in relation to "lipoid nephrosis," lymphoid hyperplasia and additional clinical syndromes.

NOTE: We are indebted to Dr. F. B. Mallory for the photomicrographs and to Miss Marion E. Lamb for technical assistance.

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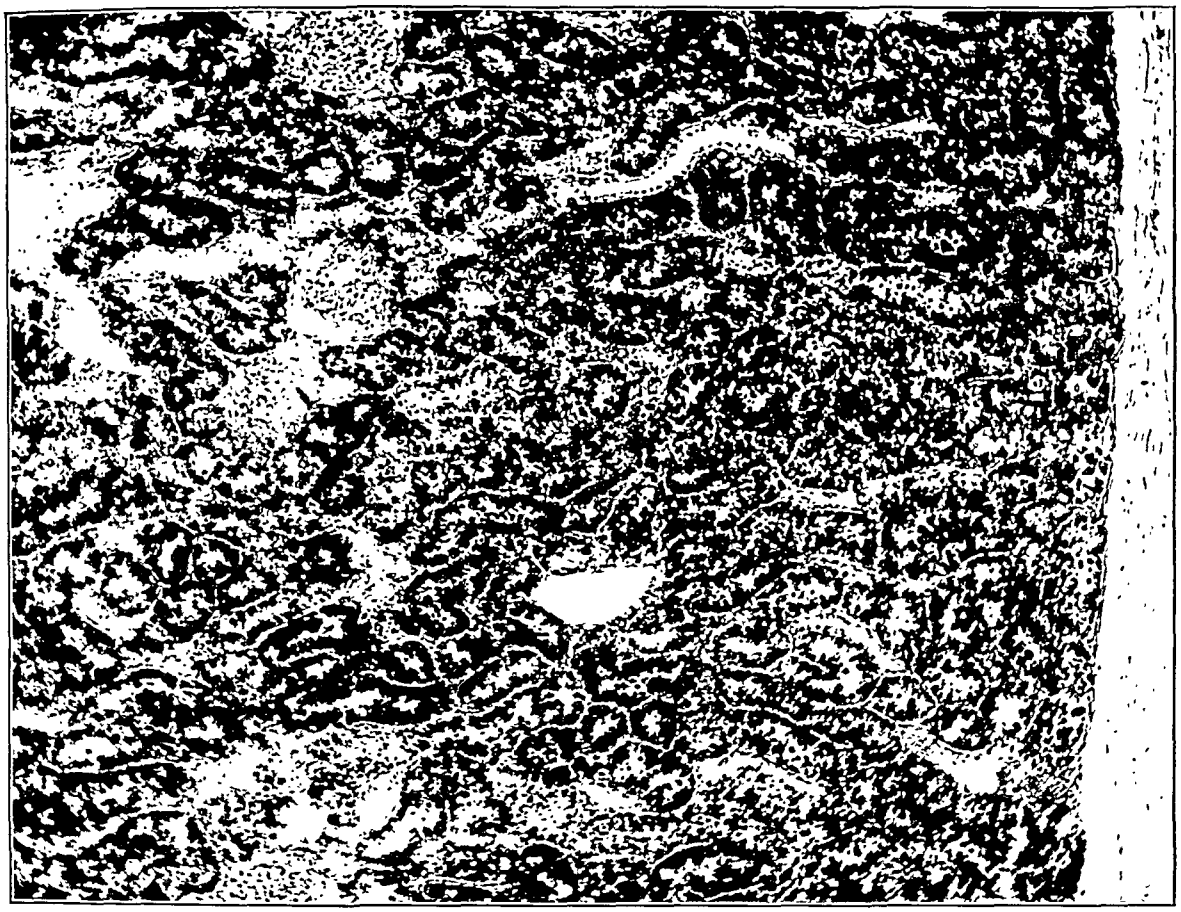
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DESCRIPTION OF PLATES

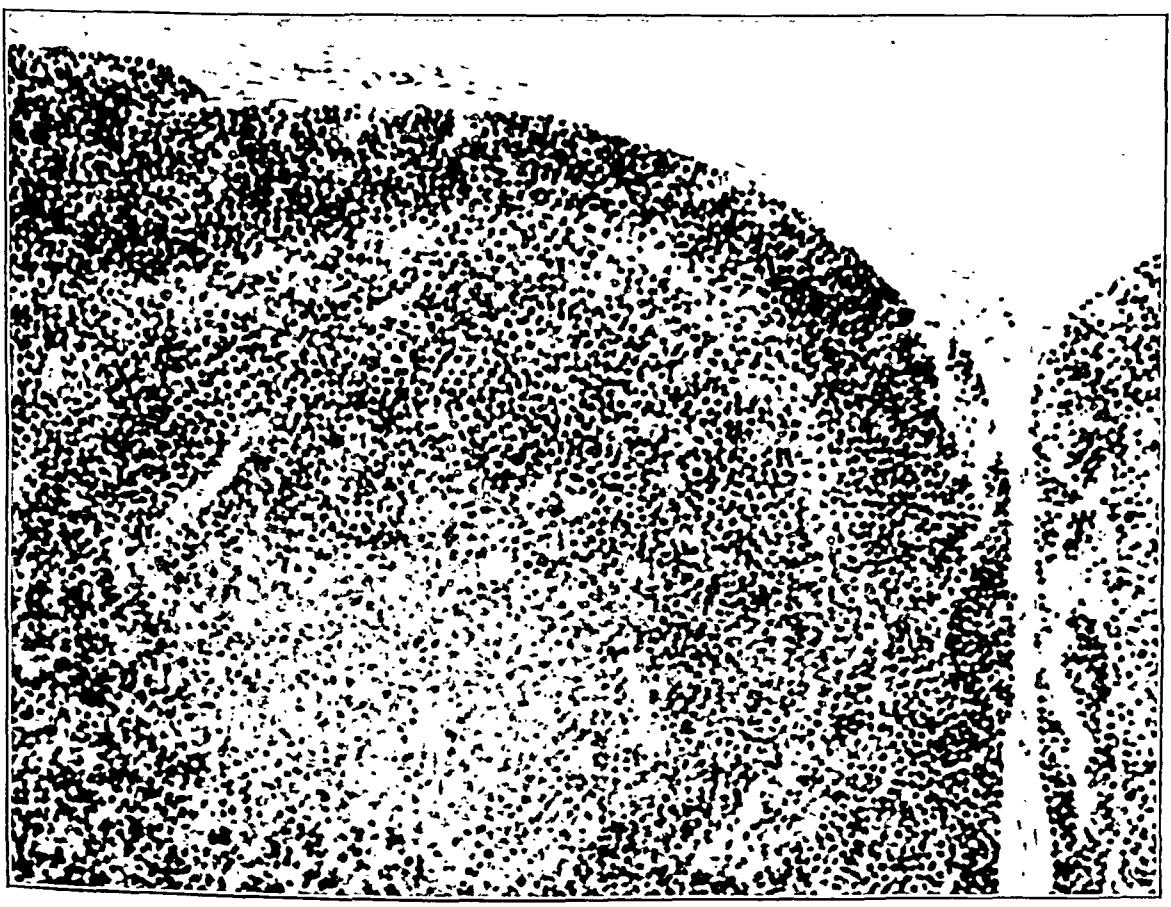
PLATE 99

FIG. 1. Photomicrograph of kidney stained with Sudan IV, showing the large amount of fat in the tubular epithelium. × 100

FIG. 2. Photomicrograph of portion of lymph node, showing hyperplastic lymph nodules and the peripheral sinus distended with mature lymphocytes. × 150



1



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MacMahon and Zwemer Pathologic Histology of Adrenalectomized Cats

CONGENITAL ANOMALIES OF THE LIVER *

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The microscopic study of a large series of livers from premature and full-term infants, infants one to several months of age, children and adults has shown clearly that congenital anomalies may occur within the liver in regard to the arrangement of the liver cells, the lobules, the intrahepatic bile ducts, and also in the mesenchymal architecture of the portal areas. The importance of these variations is probably more of embryologic or biologic interest, and yet they should be recognized by the pathologist as forming a histologic basis of some of the more unusual forms of intractable jaundice of infancy. Furthermore, they should be considered carefully both in the differential diagnosis of healed forms of cirrhosis and in the interpretation of some of the unusual histologic lesions at times seen in the fully developed liver.

EMBRYOLOGY OF THE LIVER

The development of the human liver, according to the majority of anatomists, recapitulates broadly the forms recognized in the evolution of the animal scale. The liver begins as a ventral outgrowth of the entodermal tube, a stage seen in the amphioxus in which the liver is merely a cecal diverticulum of the digestive canal. In the shark, this outpouching has developed into considerable size; it is formed of epithelial tubes with lumina, known as hepatic cylinders. In the amphibians there is further development, the cylinder and lumina are smaller, and the organ is a modified tubular gland composed of these cylinders of hepatic cells arranged around bile ducts. A similar picture is seen in the 10, 12, 14 and 16 mm. human embryo, in which the liver is composed of a network of reticulating cylinders bordered by endothelial-lined spaces, but at this stage the intrahepatic bile ducts have not yet developed.

In the further development of the embryo the tubular form is lost and smaller and smaller meshes of hepatic cells arise from these

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primary cylinders. At first these appear simply as a network of trabeculae lacking an orderly arrangement, but later they become grouped into lobular units.

There are different theories regarding the embryogenesis of the intrahepatic bile ducts. Minot [1] favored the view that they arose from the hepatic duct — "the origin of the interlobular ducts is as yet but imperfectly understood, but we may surmise that they arise as evaginations of the hepatic duct: certain it is that they are always distinct from the hepatic cells." According to Arey,[2] "at eight weeks hollow interlobular ducts appear spreading inward from the hepatic duct along the larger branches of the portal vein." Schäfer,[3] Lewis,[4] Hertwig,[5] von Meyenberg [6] and Keith [7] regard at least the smaller periportal ducts as arising from the liver cells, while von Meyenberg in his interpretation of liver cysts concluded that the large periportal ducts arose from the hepatic duct. According to Keith, "the ducts of the liver, unlike those of any other gland, arise by a secondary process. Undifferentiated cells lying along the distribution of the portal vein in the liver, group themselves into cords, develop lumina, become covered with mesodermal tissue and then form the intrahepatic bile ducts."

Lewis describes the process as follows: "The trabeculae form cords extending along the surface of the periportal mesenchyma and in them a lumen is formed. In places the cells on the mesenchymal side of the lumen are distinctly flatter than those toward the portal capillaries. In a later stage the mesenchyma has increased so that it surrounds the ducts which were seen forming along its surface. Their epithelium has become regularly cuboidal or columnar."

The commonly accepted view regarding the development of the extrahepatic biliary system is that the hepatic ducts arise within the stalks of the solid hepatic buds, that is from the anterior portion of the hepatic diverticulum; while from the caudal portion are developed the common bile duct, the gall bladder, and the cystic duct at the junction of the common bile duct and the gall bladder. Moreover, in addition to arising from different portions of the original hepatic anlage, these structures develop in different stages so that one portion may have a lumen while another is still a solid cord of cells.

The primary vessels of the liver are the two vitelline veins which form a close anastomosis with each other. With invasion by liver

tissue and the formation of sinusoids, these veins become resolved into a distributing and collecting group. The distal group comprises the system of hepatic veins, whereas the proximal portion of the two vitelline veins forms a single vessel which represents the portal vein of later stages. About the fifth week of embryonic life the left umbilical vein establishes a communication with the vascular plexus of the liver, so that placental blood as well as blood from the intestines now passes through the liver. Soon afterward, a large channel, the ductus venosus, develops between the proximal and distal groups of vitelline vessels; this conveys the larger portion of placental blood directly into the inferior vena cava.

Very early the common bile duct, the cystic and hepatic ducts are surrounded by mesenchyma. With the development of the hepatic circulation the mesenchyma spreads along the ramification of the portal vein into the substance of the liver. Periportal ducts form, and branches of the hepatic artery, together with nerves and lymphatics extend along into this loose mesenchyma so that the wide portal areas show a rather sharp contrast to the little connective tissue that surrounds the thin-walled central veins.

In brief, the development of the mammalian liver may be traced from a simple diverticulum of the foregut, through a compound tubular structure composed of reticulating cylinders, through a stage in which the liver is composed of a network of liver cell trabeculae lacking orderly arrangement, and finally into the mature form in which it is divided into lobular units. The peripheral bile ducts appear to develop from undifferentiated parenchyma cells bordering the portal areas. These form larger ducts which communicate with branches of the hepatic duct which arise outside of the liver. The gall bladder, cystic and common ducts develop independently. The mesenchyma of the portal areas extends in along the portal vein and increases with the appearance of branches of the hepatic artery, nerves and lymphatics.

In this short review of the embryology of the liver we have stressed a few of the more important developmental processes which form the basis of some of the more common congenital anomalies seen in later months.

GROSS ANOMALIES

The number of anomalies that might arise in an organ having such a complicated process of development could be very large, and probably many of the minor variations are often overlooked.

Holmes [8] describes congenital obliteration of the bile ducts as they affect the hepatic, cystic, common duct and gall bladder, and reviews the gross morphologic relation in one hundred reported cases. He favors the view that the obliteration is a developmental anomaly rather than infectious, and points out that in at least sixteen of these cases surgical relief was possible, while Thomson[9] first drew attention to the cirrhosis of the liver which may result from an obliterative process in the large biliary system.

Boyden [10] has described the gross morphologic anomalies of the mammalian gall bladder. Meckel [11] reported a case of complete absence of the gall bladder, and Kermisson and Hébert [12] collected three cases in which there was no trace of the hepatic, cystic or common ducts.

In the liver, the more common gross anomalies are irregularities in form, in the number of lobules and in the presence of cysts; while a very much rarer finding is the presence of one or more accessory livers.

HISTOLOGIC ANOMALIES

The congenital histologic variations of the liver, exclusive of those changes which occur as a result of some abnormality in the extrahepatic biliary system, may be separated into two groups: first, those in which there has been simply a retardation in the normal process of development, in which case the liver of a full-term child may structurally resemble, in part at least, the liver of a developing fetus; and secondly, those in which there is a true developmental anomaly involving either the parenchyma or the connective tissue forming the portal areas.

GROUP I. Two varieties were found that fall into this group. In one which occurred in a full-term child who lived seven months the structure resembled a network of reticulating cylinders, in which tubules of eight and ten liver cells surround large and small lumina. This simulated in arrangement the developing liver of a young mammalian embryo, or the mature liver of an amphibian.

The second example occurred in a full-term child who lived several months and was jaundiced since birth. As in the former case, the extrahepatic biliary system was normal. The liver was composed of an anastomosing network of poorly defined trabeculae of liver cells separated by wide endothelial-lined spaces. The liver cells varied in size and shape and were poorly defined, while some contained six and eight nuclei. They were arranged in single rows, sometimes in double rows with a distended bile capillary between. There was no lobulation, but here and there the parenchyma was traversed by irregular portal areas in which normal periportal ducts could be followed. The jaundice which occurred in this case probably resulted from a lack of continuity of the bile capillaries, which in turn was dependent on the disorderly arrangement of the liver cells.

GROUP II. Probably one of the commonest variations seen in the liver of newborn infants is the irregularity in the amount of mesenchyma in the portal areas. Sometimes an unusually large amount of connective tissue has grown in along the large vessels. In the infant this tissue may be arranged loosely and appears very conspicuous. In later life with condensation and increase in the number and size of the lobules it becomes a much less noticeable feature. At either age, it appears to cause no functional disturbance. Two of the cases of this type were complicated by the presence in the portal connective tissue of many blood-forming cells similar to those lying in the perisinusoidal spaces.

Another type of anomaly likewise involving primarily the mesenchyma occurred in a full-term child who lived nine weeks. This child had also been jaundiced since birth. The portal connective tissue in all portal areas was increased in amount and was very condensed. The portal vessels were numerous, small and compressed. The periportal ducts were increased in number and could be traced as double rows of cells often showing no lumen. These duct cells resembled the poorly differentiated embryonic type, and lacked the normal development into the mature cuboidal and columnar forms. It is possible that this abnormality is primarily a maldevelopment of the periportal ducts; however, the increase and condensation of the mesenchyma is such an outstanding feature that the duct anomaly is probably secondary.

The normal liver of a pig and also of a camel (Mall [13]) is divided into distinct and separate lobules by strands of connective tissue.

Occasionally the human liver may show this reversion in varying degrees. The human liver may be resolved into well defined lobules partly by rows of liver cells at the periphery which lie at right angles to the radiating trabeculae, and partly by narrow bands of connective tissue.

This division into lobules may be much more irregular and much more complete. Narrow bands of mesenchymal tissue may extend from one portal area to another dividing the parenchyma into single and compound lobules, often lacking any visible central veins. In one case of this type, in a child twenty months old showing other congenital stigmata, the peripheral connective tissue was bordered by young sprouting periportal ducts which had not become detached from the lobule by the surrounding mesenchymal tissue (Figs. 1 and 2).

This last case is of unusual interest because it shows a complete border of periportal ducts developing from the undifferentiated peripheral parenchyma cells. Bloom[14] has recently attempted to prove that the change of liver cell cords into bile duct epithelium is dependent on the former being surrounded by connective tissue. According to this author, "when the connective tissue is absent, the epithelium surrounding the lumen of the tube is continuous and morphologically indistinguishable from the remainder of the hepatic parenchyma." In the case reported here this transformation occurs in the absence of surrounding connective tissue, which suggests that some factor other than connective tissue may control the embryogenesis of these periportal ducts. Furthermore, there is evidence to show that the cells of the periportal ducts are relatively less differentiated in the infant than in the adult. In an adult, a toxin that kills off all the liver cells of a lobule leaving only the periportal ducts, results in the regeneration of cells, simulating the periportal duct cell, which apparently never form true liver cells. In an infant's liver that has suffered a similar destructive lesion, the periportal cells may regenerate in the form of tubules, resembling by the morphology of the cell and the presence of bile in the lumina, liver cells; and by the tubular arrangement of the cells, poorly formed bile ducts (Fig. 3).

There is another anomaly of the liver which occurs more frequently that is characterized by an increase in number and size of the periportal bile ducts. Many of these, even in the seven-month

fetus, are unusually dilated and appear as distended tubules and small irregular cysts. These cyst-like ducts contain eosin-staining finely granular coagulum. The cells lining the dilated ducts are small, rather uniform and cuboidal, and have little cytoplasm, whereas the nucleus is relatively large and resembles that in the liver cells. At the margin of the portal areas are ducts that are incompletely formed; the row of cells lying adjacent to peripheral mesenchyma resembles bile duct epithelium, whereas the cells toward the lobule are like liver cells and are continuous with the liver cells of the lobule.

A well marked case in an eight-month fetus showed the liver parenchyma divided up irregularly into uneven lobules by coarse bands of loose mesenchyma. The large, dilated, poorly formed periportal bile ducts were distributed along the surface of the portal connective tissue (Fig. 4). The larger portal areas contained as many as six to eight thin-walled moderately large blood vessels.

In another case, in a full-term child one month old, the many dilated ducts were less prominent. The cells lining the ducts were uniform in size and better defined, but were still relatively undifferentiated. There was more connective tissue in the former case, and most of the ducts were completely surrounded by it.

The liver of a child three months old showed a similar picture. The number of dilated bile ducts was about the same as that in the earlier cases, but with further condensation of the stroma they were less conspicuous. They did not appear to be increasing in number or in size, and the duct cells showed no mitoses. The cells were more mature and in places tended to become low columnar, with the nuclei more toward the base. Normal portal areas were seen at the margin of many of the more recently formed lobules, but the greater part of the liver still lacked normal lobulation (Fig. 5).

This type of anomaly persists with the growth of the individual. An excellent example was seen in a child 15 years of age. The large portal areas showed an increase in both number and size of the ducts as well as an increase in blood vessels and connective tissue. The epithelium of the ducts was generally of a very uniform cuboidal type, although at times it was low columnar. The presence of bile within these ducts demonstrates that they are part of the functioning biliary system (Fig. 6).

The liver of an adult, which grossly may appear quite normal, may

show this same malformation. Here, however, with the growth of liver cells and condensation of the portal connective tissue, this anomaly which formed such an outstanding feature in the fetus may appear relatively insignificant (Fig. 7).

Sometimes, as not infrequently happens both in the embryo and adult, a portion of a duct becomes detached and separated off as an epithelial-lined cyst. This may increase in size and result in compression of the surrounding stroma. The watery fluid that is secreted into the cyst may so distend the wall that with gradual thinning and elongation of the epithelial cells they resemble a delicate wall of endothelium (Fig. 8). In time the epithelium may atrophy, and then the fluid is absorbed, the walls contract, and the space becomes obliterated by fibrous tissue.

In this type of congenital malformation of the ducts the process begins with an abnormal development of the periportal bile ducts. When once formed they do not disappear but become gradually less conspicuous with the later development of the liver. Whether the anomaly is primarily the result of anomalous development of the periportal bile ducts, or whether it is secondary to an increase in mesenchyma that is commonly present, is a debatable question; however, the unusual regularity with which this anomaly is associated with the bilateral congenital cystic kidney would suggest that we are dealing with a primary developmental defect of parenchymatous origin. In the infant the liver may be normal in size, contour and color. It may cut with slightly increased resistance. Fibrous bands may be irregularly followed extending into the liver from the transverse septum. In the adult the liver may be grossly normal or filled with large and small cysts. According to Moschcowitz [15] the congenital cysts of the liver arise from aberrant ducts, one type of cyst arising from "inflammatory hyperplasia of these ducts," the other by retention of fluid as a result of congenital obstruction.

Summary

Congenital anomalies of the liver are relatively infrequent. They may involve the biliary system, the liver cells or the stroma. Several of the more common types are described and explained on an embryologic basis. One type that is characterized by an increase and dilatation of the periportal bile ducts, accompanied by an in-

crease in connective tissue in the portal areas, may persist throughout life. Associated with this type of anomaly are the congenital cysts of the liver occasionally seen at birth and also in later life. Moreover, this anomaly, with or without cyst formation, very commonly accompanies bilateral congenital cysts of the kidneys.

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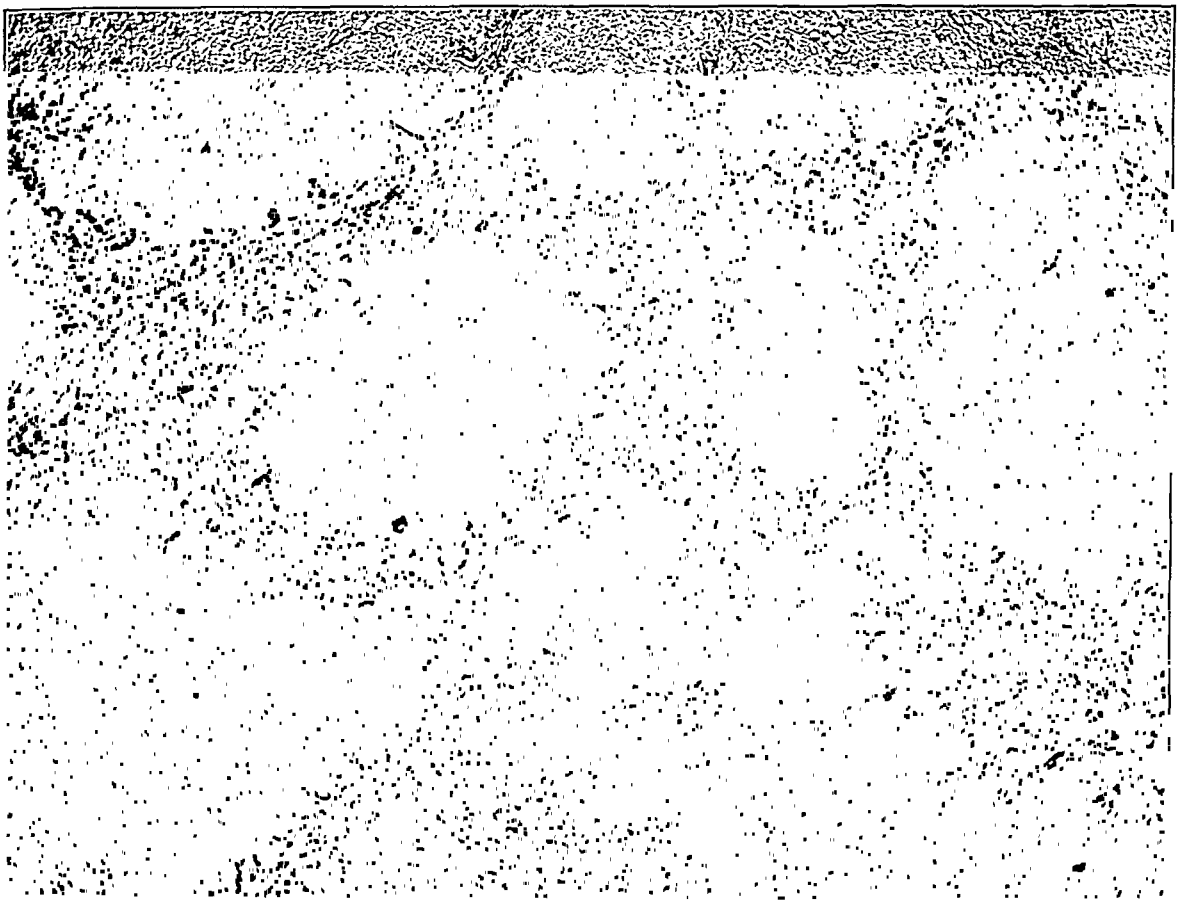
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DESCRIPTION OF PLATES

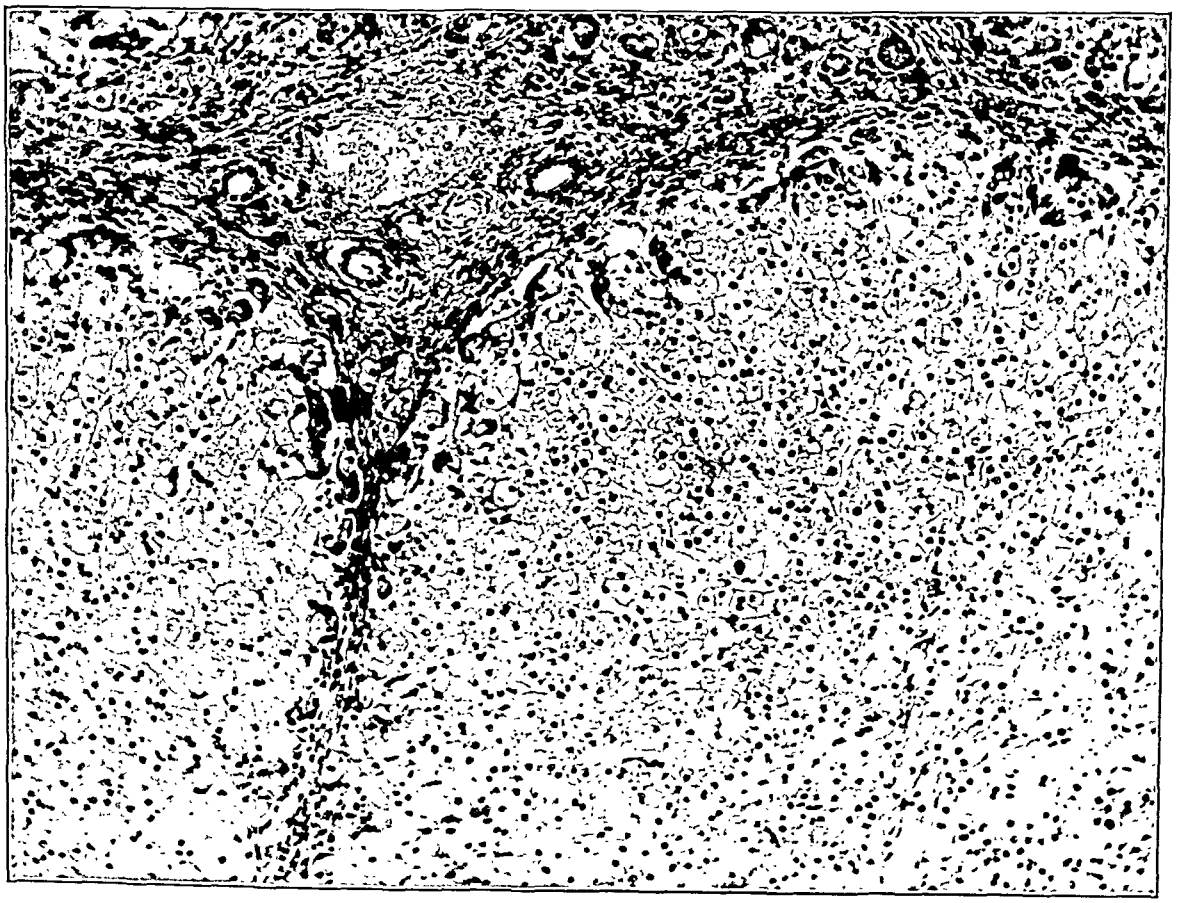
PLATE 100

FIG. 1. Photomicrograph of the liver of a full-term child who lived twenty months. The parenchyma is divided into single and compound lobules, many of which lack central veins. Developing periportal bile ducts border the periphery of the lobules. × 50.

FIG. 2. A higher power photomicrograph of the liver in Fig. 1. The portal connective tissue contains well formed vessels and bile ducts. The small embryonic periportal bile ducts lie at the periphery of the lobule. The liver cells lack uniformity. The trabecular arrangement is lost, and bile capillaries are indistinct. × 150.



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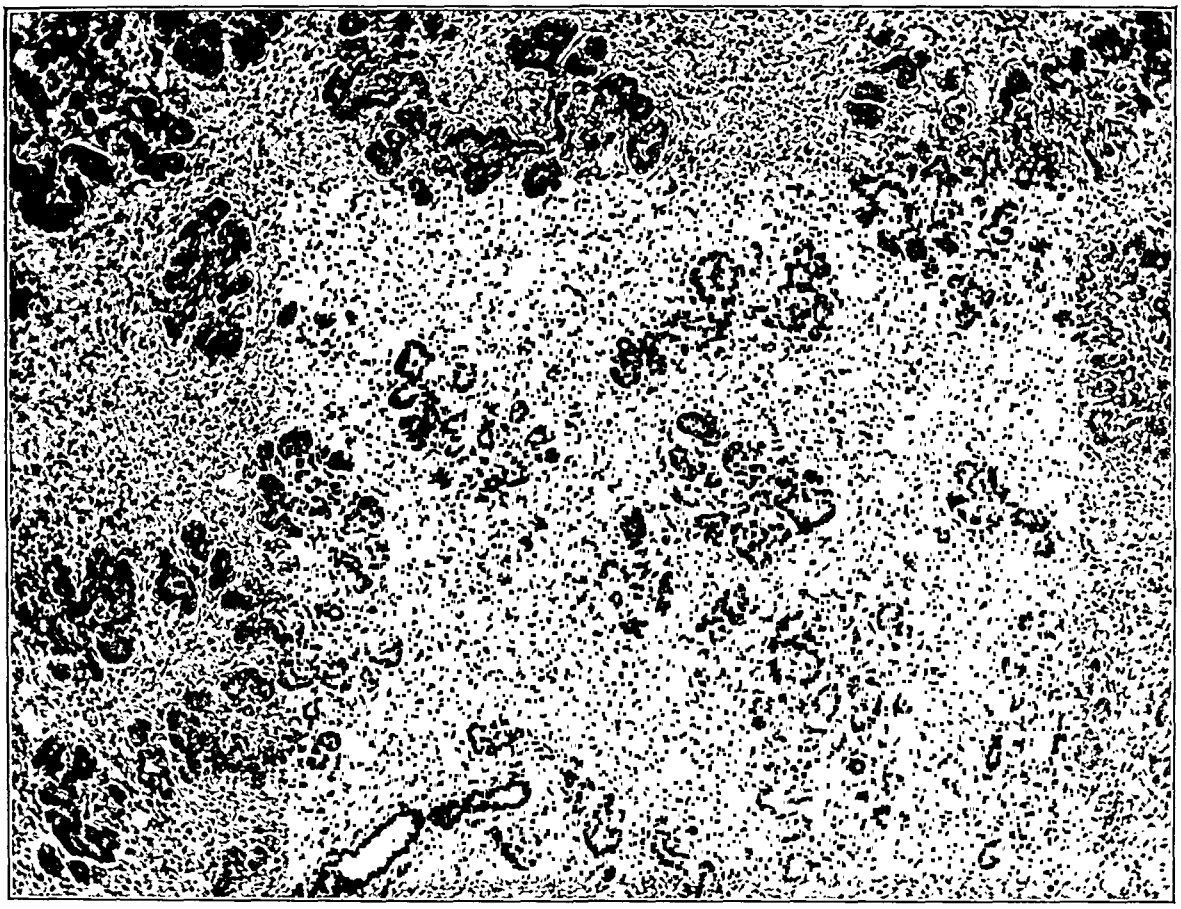
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MacMahon Congenital Anomalies of Liver

PLATE 101

FIG. 3. Photomicrograph of the liver of a three-months-old child showing the healing stage of an acute toxic hepatitis. The liver cells of the lobules have been killed. The trabecular spaces are filled with endothelial leucocytes. Regenerating periportal bile ducts border the portal areas. The morphology and the presence of bile in bile capillaries bordered by the cells suggest that they are liver cells, whereas their arrangement more closely resembles poorly formed bile ducts. × 100.

FIG. 4. Photomicrograph of the liver of an eight-month fetus. Large dilated cyst-like periportal ducts border the peripheral mesenchyma. The liver cells are separated by wide endothelial-lined sinusoids and are arranged as a loose network of interlacing trabeculae. There are many foci of cells showing hematopoiesis in the perisinusoidal spaces. × 100.



3

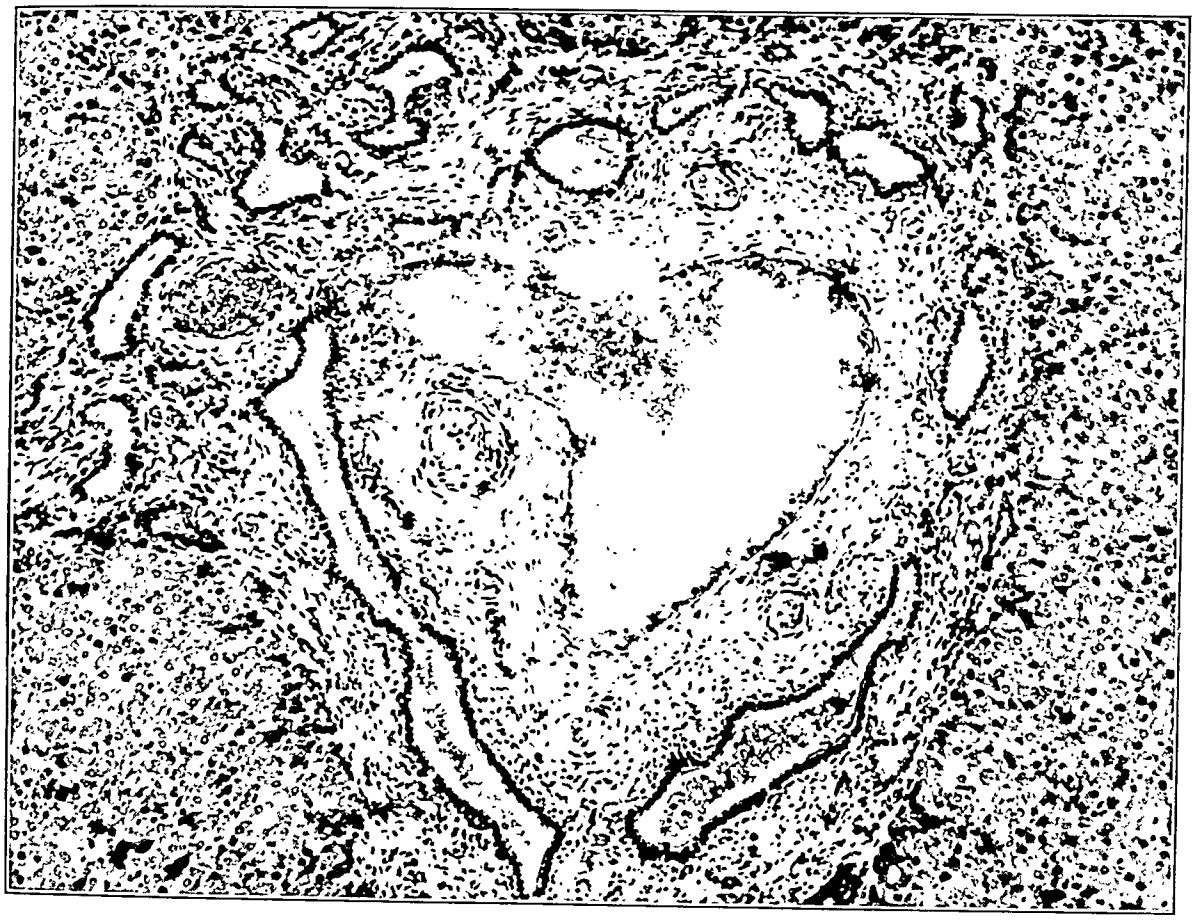


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PLATE 102

FIG. 5. Photomicrograph of the liver of a full-term child who lived for three months. The many dilated bile ducts stand out in sharp contrast to the moderately condensed stroma. The cells lining the bile ducts are uniform in size and more differentiated than in the preceding case. × 150.

FIG. 6. Low power photomicrograph of the liver of a child 15 years of age. The larger portal areas contain many dilated bile ducts. The presence of bile in some of these suggests that they form part of the functioning biliary system. × 50.



5

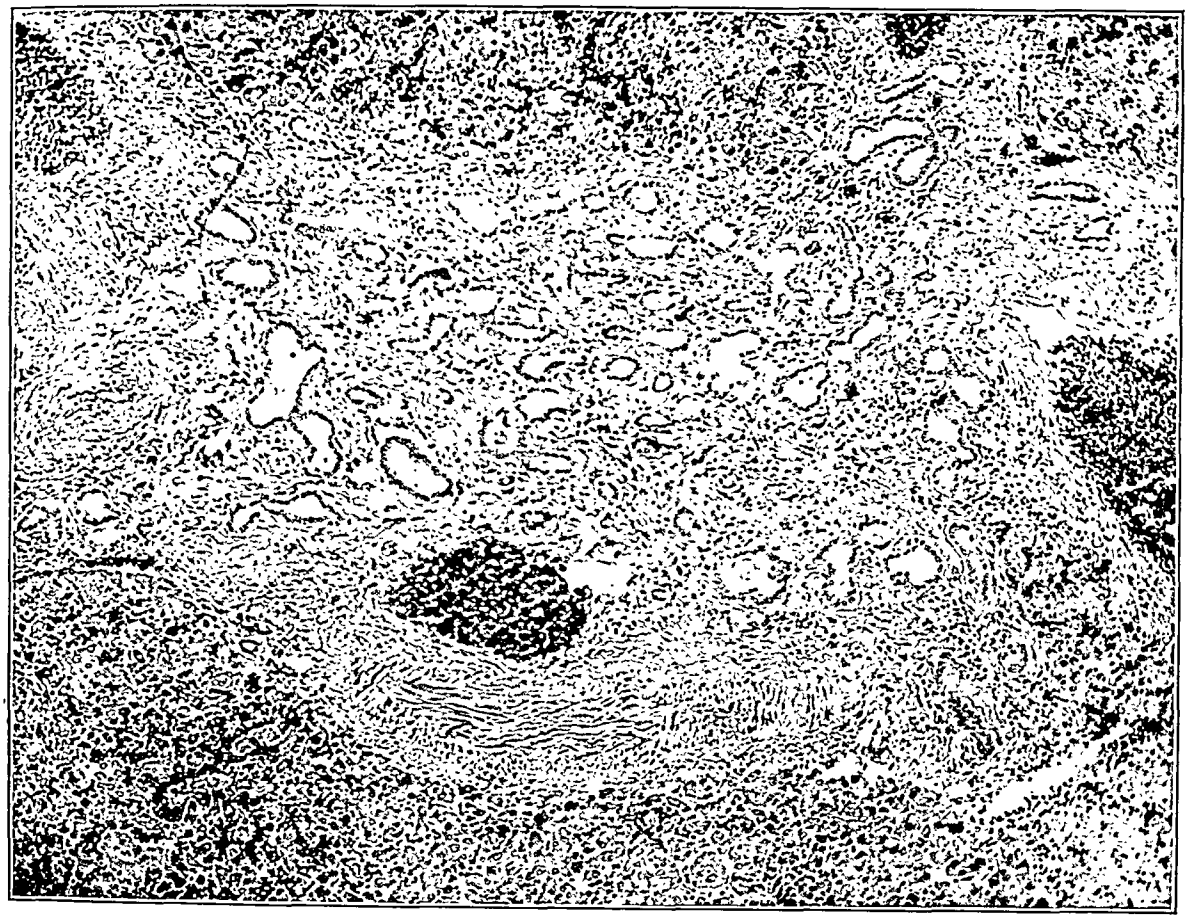


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PLATE 103

FIG. 7. Photomicrograph of the liver of an adult showing the same anomaly of the bile ducts. They are increased in number, moderately dilated and lined with a uniform cuboidal type of epithelium. The connective tissue stroma, as in the earlier cases, is also considerably increased. × 100.

FIG. 8. Photomicrograph of the liver from an elderly male. It shows the same bile duct anomaly. Here some of the ducts have become isolated, and as cyst-like spaces are distended with clear fluid. The bile duct epithelium is extremely compressed. The unusual amount of connective tissue surrounding the cysts suggests an old healed inflammatory lesion. × 50.



7



8

MacMahon

Congenital Anomalies of Liver

SCIENTIFIC PROCEEDINGS OF THE TWENTY-NINTH ANNUAL MEETING OF THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

CHICAGO, ILL.

MARCH 28 AND 29, 1929

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

REPORT OF A CASE OF PEL-EBSTEIN'S DISEASE OF TUBERCULOUS ORIGIN. Elise S. L'Esperance, New York City.

(Abstract not received.)

SKIN REACTIONS IN SPRUE PATIENTS TO VARIOUS FOOD PREPARATIONS. Charles Weiss and (by invitation) Oscar Costa-Mandry, St. Louis, Mo.

Abstract. An investigation was made to determine whether the untoward gastro intestinal reactions observed in sprue patients (diarrhoea, tympanites, flatulence, etc.,) after eating certain types of food might be of an allergic nature. A careful survey was made of the foods commonly eaten by the people of Porto Rico, and representative types were selected as antigens for intracutaneous tests. These included *proteins* prepared from rice, beans, pork, cod-fish, cocoanut, etc.; *sugars* (saccharose and fructose); *fats and oils* (lard, butter, etc.,); *starches* prepared from rice, potatoes, beans, etc.; and local spices and coffee.

Our results were clearly negative and do not lend support to the hypothesis that the untoward digestive symptoms of sprue patients are due to specific hypersensitiveness to certain food products.

THE ANTIGENIC PROPERTIES OF THE PROTEIN FROM TUBERCULIN. J. H. Lewis and (by invitation) F. Seibert, Chicago, Ill.

Abstract. The proteins used in these experiments were obtained from 6 to 8 weeks cultures of avian, bovine and human tubercle bacilli and timothy bacilli grown on Long's synthetic non-protein liquid medium, and were isolated either by precipitation with ammonium sulphate or by ultrafiltration. The processes used in either case were such that denaturation was prevented. The proteins were readily soluble in water, forming perfectly clear solutions. While they were not crystallized proteins they were the starting point where Seibert began her crystallization of the tuberculin protein. In doses of 0.1 milligram (the smallest amount tested) they were actively anaphylactogenic as proved by intravenous injections of 10 to 15 milligrams into sensitized guinea pigs, by the uterine strip method, and by the bronchospastic method of Lewis and Koessler. Cross-sensitization tests (intravenous and uterine strip) made with avian, bovine and human tuberculin proteins showed that these three proteins give cross-reactions but are distinguishable from each other by desensitization tests. Uterine strips from guinea pigs sensitized with the tuberculin proteins do not react to the protein from timothy bacilli filtrates, and *vice versa*. Guinea pigs injected with the human tuberculin protein produced high titers of precipitins. The sera containing such precipitins could passively sensitize normal guinea pigs in doses as small as one cubic centimeter. Tuberculous guinea pigs died from acute anaphylactic shock when the tuberculin protein was injected intravenously. With the bronchospastic and uterine strip methods it was shown that this true anaphylactic sensitization developed about six weeks after infection. Sera from tuberculous guinea pigs and from some humans with tuberculosis contained high titers of precipitins, but, in contrast to precipitating sera from

normal immunized guinea pigs, they were not able passively to sensitize guinea pigs.

Discussion

(Dr. A. F. Coca, New York City.) The most surprising thing about this very interesting study is the lack of specificity which seems to be shown in some of the earlier experiments, although Dr. Lewis does give one experiment, at any rate, that demonstrates a certain specificity of the protein of the timothy bacillus.

With respect to bovine and human tuberculin, I am not sure whether there is a definite, specific difference in the tuberculin active substance; but between avian and human tuberculin, there is a distinct impression from the experiences of Lowenstein that they are specifically different as tested in the infected individual.

Dr. Wollstein says that recently she has had a considerable series of experiments of that kind carried out on children in New York, and found some of them (something over 100 cases) positive to the human tuberculin, but negative to any concentration of avian tuberculin. There may be some question as to whether these experiments of Dr. Lewis' need to coincide with those carried on in the human being. At any rate they seem to disagree, because I understand that Dr. Lewis believes that he has isolated here the particular substance that is responsible for the tuberculin reaction. There is a discrepancy here that evidently needs further study with reference to the interesting report we had yesterday by Dr. Dienes, who, no doubt, would have something further to say along that line.

(Dr. E. L. Opie, Philadelphia.) I should like to ask Dr. Lewis if these guinea pigs gave skin reactions to the protein that he used.

In an effort to correlate anaphylactic hypersensitiveness and the tuberculin reaction Baldwin and Austrian failed to obtain skin reactions with tuberculin prepared by the usual methods, though they obtained anaphylactic shock by injection of tuberculo-protein into the blood stream or into the central nervous system. They have emphasized the distinction between anaphylactic hypersensitiveness to tuberculo-protein and cutaneous and toxic sensitiveness to tuberculin.

(Dr. Stuart Mudd, Philadelphia.) I think the apparent discrepancy Dr. Coca has pointed out can be explained. Extensive studies of the serology of acid-fast bacteria have shown that avian tubercle bacilli give strong cross-reactions with bovine and human strains; indeed reciprocal adsorption tests may be required to differentiate the avian and mammalian strains with certainty. On the other hand certain saprophytic strains, of which "timothy" is no doubt one, do not cross-react with mammalian tubercle bacilli.

I was surprised though, if I understood correctly, that there should be a distinction between bovine and human tubercle bacillus proteins, because all the work that has been reported and all we have done has failed to show any serological distinction between the human and bovine strains.

(Dr. E. R. Long, Chicago.) The results just brought out are much more striking than those secured by Dr. Baldwin some years ago, which are considered the pioneer work on this subject. It should be pointed out that Dr. Baldwin used a water extract of dried tubercle bacilli, and in the drying process there is no doubt that a considerable amount of change took place. There is much loss of biological activity in drying. These results are probably so much more strik-

ing than Dr. Baldwin's because the materials were subjected to chemical methods not likely to lead to change in the character of the proteins.

I am much interested in what Dr. Coca and Dr. Mudd had to say about the relationship of these microörganisms. I might recall some work which I did some years ago on the tuberculin reaction. I found that animals that were sensitive to human tubercle bacilli and corresponding tuberculin were also sensitive to other organisms of the acid-fast group but not to the same degree. My own feeling is that probably not exactly the same protein, but related proteins are present in the bacilli of this group.

(Dr. E. Pribram, Chicago.) I should like to know whether these animals became immunized because of the high character of the protein and become less sensitive, which affords a protection from infection with the tubercle bacilli.

(Dr. H. Swift, New York City.) I should like to ask whether the skin reactions (just amplifying Dr. Opie's question) were of the immediate type occurring within the first two or three hours or whether they were the late tuberculin type of reaction.

(Dr. L. L. Dienes, Asheville, N. C.) With preparations similar to those used by Lewis and Seibert, we obtained abundant antibody production only in tuberculous guinea pigs, not in normal ones. Complement fixation antibodies were produced in larger amounts than precipitins. In the filtrates of synthetic media we found only the proteins precipitable with acid, antigenic *in vivo*, and this protein fraction is a different antigen from the fraction non-precipitable with acid. We have to pay special attention to the fact that certain protein preparations produce antibodies in abundance toward carbohydrate precipitable substance. The antigenic effect *in vivo* of different preparations varies to a great extent, and possibly this is the reason our observation does not entirely correspond to that of the authors.

(Dr. Lewis, closing.) We studied cross-anaphylactic reactions with two methods, the intravenous injection method and the uterine strip method. With the intravenous injections into sensitized animals it was impossible to distinguish between the three types of tubercle bacillus proteins, since animals sensitized to one died equally rapidly when injected with either one of the other two. We did not try to desensitize living sensitized guinea pigs with non-fatal doses of protein, but did make desensitization experiments with the uterine strip method which lends itself particularly well to this purpose. There is a similarity between the three tubercle bacillus proteins because a uterine strip sensitized to one will react to either of the other two, but after such a reaction there always remains some sensitization to the homologous protein. In other words, the three proteins are closely related but are not identical, since one will not desensitize to the other. Whether the bovine protein is more closely related to the human protein than avian, we cannot say from experiments made so far.

We did not make comparisons of the tuberculin test and the anaphylactic reaction in tuberculous animals because we wanted to avoid any anaphylactic desensitization that might occur from the injection of small amounts of tuberculin.

In animals given several injections of as large quantities of tubercle bacillus proteins as were not fatal, a suggestion of a skin reaction could be elicited which resembled a tuberculin reaction. The impression is given that if the protein was less toxic so that larger and more frequent injections could be given, a degree of skin sensitization could be obtained which would simulate very closely a true tuberculin reaction.

We did not try to study the protection from infection with tuberculosis that injections of these proteins afforded. We were not interested in this problem at that time.

In answer to Dr. Swift's question about the immediate reaction, we considered only as positive anaphylactic reactions those animals that gave an immediate, acute reaction and disregarded those reactions which were delayed and which were fatal the next day, because we could not be sure that the latter type of reactions was due to anaphylaxis or to the primary toxicity of the proteins. We used the same criteria that we use in anaphylactic tests with other proteins. We made no attempt to study the relation of carbohydrates to these experiments. We were aware at all times that our preparations contained carbohydrates. Dr. Seibert states that tubercle bacillus proteins are freed of carbohydrates only after repeated crystallizations. As the immune reactions of these proteins after purification by crystallization will be studied, some facts will be obtained on the rôle of carbohydrates.

ON THE MECHANISM OF OPSONIN AND BACTERIOTROPIN ACTION-ANTIBODIES IN EXPERIMENTAL TUBERCULOSIS IN THE RABBIT. Baldwin Lucké, Morton McCutcheon, Max Strumia, Emily B. H. Mudd and Stuart Mudd, Philadelphia, Pa.

Abstract. Last year we reported that whenever in our experiments acid-fast bacteria were prepared by serum for phagocytosis certain definite changes in the surface properties of the bacteria could be demonstrated; that, furthermore, whenever these bacterial surface changes were found, except under certain artificial conditions, the bacteria were prepared correspondingly for phagocytosis. The bacterial surface changes correlated with the increased phagocytosis are increased cohesion, decreased surface electric potential difference and decreased wettability by oil. In the present experiments we have again found this correspondence between the surface properties and the phagocytosis of bacteria under a set of conditions not hitherto tested, namely experimental tuberculosis in rabbits.

In addition to the above, certain interesting findings concerning the development of antibodies in tuberculosis have come out of those experiments. Three groups of rabbits were infected with virulent tubercle bacilli. Other groups of rabbits had been inoculated six months earlier with 0.001 mg. of virulent human tubercle bacilli; the resulting tuberculosis had largely regressed, leaving only residual foci in the lungs and occasionally in other organs. These second groups of rabbits were reinfected with virulent tubercle bacilli at the same time and with the same dosages as the primarily infected animals. This work was done by Dr. Max B. Lurie. The titers of the sera of both primarily infected and reinfected rabbits were then followed by resuspension, cataphoresis and phagocytosis.

There was found a slight and slowly developing production of antibodies in first infection rabbits which showed a moderate and slowly developing resistance to the infecting tubercle bacilli, and a prompt production of antibodies in quantity in the reinfection animals, which were highly resistant to the reinfecting organisms. This association of heightened resistance with increased capacity for antibody production in reinfection animals suggests, without proving, however, a rôle of antibodies in immunity to tuberculosis.

The development of antibodies causing bacterial surface changes and phagocytosis occurred, within the experimental error, simultaneously.

Discussion

(Dr. E. L. Opie, Philadelphia.) One phenomenon that has been brought out by this study is of especial interest, namely, the capacity of an animal previously immunized to form antibodies with more rapidity on the second immunization than on the first. As Dr. Mudd has pointed out, this has been observed with a number of microörganisms, first by Dr. Cole with the typhoid bacillus. Even after the complete disappearance of antibodies, a second immunization would cause them to reappear more rapidly than the first immunization. This phenomenon suggests that protection is dependent on factors more fundamental than antibody formation.

(Dr. E. R. Long, Chicago.) Am I right, Dr. Mudd, in assuming that the phagocytosis was carried out entirely with polymorphonuclear leucocytes? I wonder if it would not be possible to carry out this test with the large mononuclear cells. While it is probably true that the same mechanism is concerned in the phagocytic activity of each cell, and also true that the polymorphonuclear is of more importance in tuberculosis than is generally conceded, I should think if the experiment could be carried out with the large mononuclear cell that it should be done.

(Dr. Mudd, closing.) I can only agree with Dr. Long. Dr. Opie has brought out the same point repeatedly. That is an early item on our future program. This study has been primarily an effort to get light on the mechanism of opsonin and bacteriotropin action. Immunity in tuberculosis has been a secondary, rather than a primary, concern. Of course, you are quite right about the importance of studying phagocytosis by monocytes.

STUDIES ON THE MECHANISM OF THE REACTION OF ANAPHYLAXIS. Noble P. Sherwood, and (by invitation) Paul E. Davis and Raymond A. Schwegler, Lawrence, Kan.

(*Abstract not received.*)

CROSSED ANAPHYLACTIC RELATIONSHIP BETWEEN HORSE DANDER AND HORSE SERUM. Bret Ratner and (by invitation) H. L. Gruehl, New York City.

Abstract. The crossed anaphylactic relationship between horse dander and horse serum was studied in the guinea pig. Animals were sensitized to horse serum and cross-shocked with horse dander extract. Other animals were sensitized with horse dander extract and cross-shocked with horse serum. The above crossed reactions were further carried out using the Schultz–Dale method. In other groups, animals were sensitized by the nasal inhalation of dry horse dander in specially constructed dust chambers. The cross-shock injection was with either horse serum or diphtheria antitoxin.

The results obtained show a definite crossed relationship to exist. However, animals sensitized with horse dander extract more readily exhibited the crossed relationship when shocked with horse serum than did animals sensitized with horse serum and shocked with horse dander extract. Animals in which respiratory anaphylaxis was produced by normal inhalation of horse dander could be shocked with an injection of horse serum or diphtheria antitoxin.

Discussion

(Dr. N. P. Sherwood, Lawrence, Kan.) I should like to ask Dr. Ratner what amount of serum he used in the bath. I also am wondering about this six

minutes that he mentioned as elapsing between the time he added horse serum to the bath and the response. Such a time element I have never seen where I would call it a specific response. It might be, I presume, but I have never seen a response coming on as a specific response after six minutes.

Spontaneous contractions I have observed often in an irritable horn. If I had just added something before, then I would apparently have a positive reaction. Sometimes they come on after a considerable period.

With regard to the dosage he adds to the bath, I think more errors are included in this smooth muscle work because of not determining the toxic dose, and not realizing that it will respond to it tremendously. For example, if you take ten guinea pigs to determine the non-toxic dose of antigen to be used in the bath, and test out the normal pigs, you will find that perhaps one of the ten will respond to 6 cc. and not 5 cc., whereas another one of the ten pigs will respond to 1 cc. and above. If you took as the control pig, the pig that responded to 6 cc. and added 2 cc., you would think you had a non-toxic dose. It is imperative in this case to rule out the toxic reactions because it is impossible to determine by their appearance whether they are toxic or anaphylactic.

(Dr. W. H. Welker, Chicago.) I should like to ask Dr. Ratner how he explains the cross-reaction. The dander protein seems to be entirely different from the blood protein. In one case of extreme sensitization with dog dander, we had occasion to study this point. The water-soluble protein obtained by washing dogs gave a marked skin reaction. When immunization was carried out, the simple moisture that was on the needle caused a severe reaction. We were unable to get the slightest response on the part of any of the blood proteins of the dog. Does Dr. Ratner believe that in the case of this horse dander cross-reaction enough of the serum protein has permeated through the skin so that cases which are extremely sensitive to the serum proteins might give this response, and those others that are not particularly sensitive are not sufficiently sensitive to respond to such an extremely small amount of blood protein?

(Dr. Ratner, closing.) In answer to Dr. Sherwood, we used the 250 cc. in the bath. The question of delayed reaction, I think, is quite in order in so far as we get delayed reactions in anaphylaxis depending to a great extent on the solubility of the substance used. It is possible that the common substance in our solution had to find its way out of the complex protein combination, which may account for the somewhat delayed reaction.

As Dr. Lewis showed a number of years ago, when you use small amounts of protein combined with large amounts there is a masking of the lesser portions, so with our solutions there are present many more proteins than would be evidenced by the common factor.

The question of toxicity, I suppose, is a very classic question. We have made innumerable controls, and we do not use anywhere near the toxic dose. One cc. is the amount we use, and that amount has never produced primary toxicity in our hands.

In answer to Dr. Welker, the question of serum getting into the dander has been raised. Dr. Park particularly called my attention to that. It has been possible that in currying the horse, horse serum does get in. We used varying types of horse dander over a period of four years, getting them at various times of the year, and we believe if this is a factor, the same factor holds true with the human problem.

We believe, however, that there is a common substance, probably a globulin, for anaphylactic death was obtained with antitoxin. We used the preparation

obtained from the New York Department of Health which is supposed to contain only pseudoglobulins of horse serum.

ANTIGENIC RELATIONSHIPS OF A STRAIN OF EBERTHELLA PARADYSENTERIAE AND A STRAIN OF SALMONELLA MORGANI ISOLATED DURING AN EPIDEMIC OF SUMMER DIARRHOEA. G. M. Mackenzie and (by invitation) Louise N. Batt, Cooperstown, N. Y.

Abstract. During a small epidemic of dysentery characterized by blood and mucus in the stools, five cultures of an organism belonging to the paradysenteriae group (closely related to the Flexner type) and four cultures of organisms of the *B. Morgani* group were isolated. By fermentation reactions, agglutination and reciprocal absorptions the five paradysenteriae cultures were found to be antigenically identical. The four cultures of *B. Morgani* no. 1 were found by the same methods to represent three antigenically different varieties. Reciprocal absorption tests revealed an antigenic relationship between one strain of *B. Morgani* no. 1 and the paradysenteriae strain, and a common antigenic component. Cultivation of a single cell culture of the paradysenteriae strain in 5 per cent serum (homologous) broth with transfers every five days resulted in the appearance of two stable variants. One variant is inagglutinable in its homologous serum and in all other serums tested. The other variant agglutinates to titer in serum prepared from the mother culture and in *B. Morgani* serum, but does not agglutinate in any of the other heterologous serums available. Reciprocal absorption tests with the second variant, its homologous serum, *B. Morgani* and its homologous serum, show a common antigenic component. Other modifications of the mother strain apparently permanent in the variant are altered appearance of the colonies, acquisition of the capacity to form indol and to ferment maltose and saccharose. The possible bearing of these results on the pathogenicity of *B. Morgani* and the relationship between *B. paradysenteriae* and *B. Morgani* are discussed.

CINEMA DEMONSTRATION OF THE EFFECT OF FILTRATES OF CULTURES OF THE COLON-TYPHOID GROUP OF ORGANISMS ON THE MOVEMENT OF RABBIT INTESTINES. E. E. Ecker and (by invitation) M. A. Biskind, Cleveland, O.

REFINED ANTI-POLIOMYELITIS. Elliot R. Weyer (by invitation), William H. Park and Edwin J. Banzhaf, New York City.

Abstract. Landsteiner and Levaditi discovered in 1910 that monkeys which had recovered from poliomyelitis had neutralizing bodies for the virus in their blood. Netter showed that the blood of convalescent human cases also contained them. Netter in 1910 claimed that the injection of convalescent serum into cases of poliomyelitis even as late as twenty-four hours after the development of paralysis was beneficial.

The thought that horses might be immunized so as to furnish a plentiful and reliable supply of potent serum occurred to several. In 1910 Flexner tried to immunize a horse by injecting it repeatedly with an emulsion made from the potent cord and brain of the monkey. He tried but one horse and with disappointing results. He states "the blood serum of a horse after many months of treatment had no restraining effect on the virus either *in vitro* or within the body." In 1916 Neustaedter and Banzhaf made a new attempt to produce a potent horse serum. They first tried to prepare a vaccine by digesting the in-

fected cord and brain substance with trypsin. This was unsuccessful. They then changed the vaccine. A 5 per cent emulsion of the cord and brain substance of monkeys was prepared and filtered through paper pulp. This material was injected every two days. The subcutaneous injections beginning with 50 cc. were increased so that the eleventh injection was 300 cc. The horse then for the first time seemed decidedly ill. A bleeding was taken. The horse died the next day. This serum had decided antivirus potency. A control monkey given 0.5 cc. per cent emulsion of virulent brain substance died on the seventh day of poliomyelitis. Another monkey receiving the same dose was given twenty-four hours later 3 cc. of the serum intraspinally and 17 cc. intramuscularly. These injections were repeated on the two following days. The monkey remained well. A monkey in which the injections were begun forty-eight hours after the inoculation was not protected. Another monkey received an inoculation into the brain of a fatal dose of virus mixed with 0.6 cc. of the horse serum. This monkey survived. At the time and later, this serum was used in a number of human cases with doubtful success.

In 1917 Pettit, in the Pasteur Institute, reported that he had succeeded in developing a potent serum in sheep and, in 1919, in horses. He states that he was unable to estimate the potency of the serum because of lack of monkeys. The serum was used rather extensively in France with apparent success. Even late cases were thought by Stefanopoulo to be benefitted.

Stimulated by the good reports of the use of convalescent serum in early human cases recently reported by Aycock and having a large amount of virulent monkeys' brains on hand, we began again the extensive treatment of a horse, giving the injections of the emulsion every few days intravenously. After four months of injections we allowed the horse to rest for six weeks and then took three full bleedings. To our surprise we found the serum of the first two bleedings to be somewhat more potent than the average human serum from convalescent human cases.

One of us, Banzhaf, took two liters of the serum and refined it in the following manner: To the serum he added an equal quantity of water. The diluted serum was saturated with sodium chloride. The resulting precipitate was filtered off. To the clear filtrate a saturated solution of ammonium sulphate was added up to 33 per cent to precipitate the pseudoglobulins. This precipitate was pressed to remove the excess of salts and then dialyzed free from the remaining salts. To the dialyzate was added 1 per cent of sodium chloride and trikresol as a preservative. The globulin solution was then finally cleared by passing through paper pulp and Berkefelds. The 2000 cc. of the serum yielded 350 cc. of globulin solution. Fifty cc. of this concentrated pseudoglobulin solution was added to a quantity of distilled water as is done in refining the antipneumococcic serum with the hope of making a further purification. As will be seen in the figures given on the slides, the first step in refining was successful but not the second step.

(Dr. A. B. Wadsworth, Albany.) I presume you have not had a chance to determine the length of time the serum retains its potency or whether there is any loss, or a lack of stability due to the concentration. Sometimes these concentrated products are not quite as stable as the original sera.

(Dr. Weyer, closing.) So far we have carried on the test on serum stored for only one month. In that time it apparently had lost nothing, so it seemed that we had a stable, refined serum as far as any serum could go.

THE LYMPHATIC TUMOR REGISTRY PROGRESS REPORT. G. R. Callender, Washington, D. C.

Abstract. The following case of general lymph node enlargement terminating in a sarcomatous type of Hodgkin's disease represents one of the types which is of especial value to the study for which the registry of lymphatic tumors was instituted by the American Association of Pathologists and Bacteriologists and is presented with a view to stimulating interest in that registry.

Patient born in August, 1889. Mother and two brothers have had tuberculosis but are living. Acne vulgaris of face at age of 20 to 26 which was much improved by X-ray treatment in his 26th year. In this year he noticed enlarged nodes in his axilla and groin. These were not tender or painful. At the age of 31 he had an attack of acute gastro-enteritis. At the age of 32, there was noticed a general glandular enlargement, the largest node being in the left groin. This was stated to be of the size of an English walnut. This node was removed for examination. Spleen was not palpable at this time. Many pathologists have seen this node, and the majority have considered that it was not a tumor. There appeared to be an increase in number of the lymph nodules (follicles) which varied markedly in size, and there were moderately numerous mitotic figures. There were no cells of the Reed type. The cells of the follicles were closely packed, fairly uniform in size and stained deeply. The blood was reported normal at this time. X-ray treatment reduced the size of the nodes.

At the age of 35 he had a severe attack of gastro-enteritis, cause undetermined. At this time there was greater general glandular enlargement than at the age of 32, and the spleen extended to the iliac crest. X-ray treatment reduced the nodes which did not enlarge again and decreased the size of the spleen. There was a severe prostration following this X-ray therapy from which he did not recover for several months. The nodes removed at this time are said to have been like those of the biopsy at the age of 32. One of them was reported to show tuberculosis, but this has not been verified. At the age of 37 an attack of intestinal indigestion lasted three days. At the age of 38 he was in quarters five days with "influenza."

His final illness began in June, 1928, at age of 39 years. The onset was sudden with temperature 103° to 104° F. There were dull aching pains in the left lumbar region. He was dull, and his sleep was stuporous. He partially recovered from this acute phase and went on leave but was forced to return to the hospital because of irregular weakness in his extremities and back pain. He gradually failed, showing symptoms of brain and cord involvement and died in October, 1928.

At necropsy the superficial nodes were not palpable. The spleen and deep abdominal nodes and thoracic nodes were enlarged. The histological picture is that of a very cellular, invasive Hodgkin's disease, with vascular invasion.

During the final illness the lymphocytes in the peripheral blood varied in number, the lowest being 22 per cent with 5650 leucocytes; the highest 61 per cent with 6300 leucocytes.

Discussion

(Dr. J. Ewing, New York City.) The work of the Lymphatic Tumor Registry seems to be very much needed in this country, and indeed in this Association. Quite variable views have been expressed by members of this Association regarding the diagnosis and prognosis of many cases sent to the Registry. The

existence of the group of lympho-epithelioma metastatic in the lymph nodes is not generally recognized, and these cases are often mistaken for lymphosarcoma, from which they may be very difficult to distinguish. Variations in the structure of Hodgkin's nodes are numerous and difficult to trace and evaluate. We all have to admit that our knowledge of many lesions in lymph nodes is quite inadequate to enable us to make a safe diagnosis and prognosis. I believe that a Registry of Lymphatic Tumors is more important practically than the Registry of Bone Sarcoma. I trust that the members of the Association will take the little time and trouble necessary to register all their significant cases, so that within a reasonable period the Registry will contain material enough to settle many difficult questions of diagnosis and prognosis.

(Dr. H. G. Wells, Chicago.) In your request for material of which we have an early specimen concerning which there has been doubt, do I understand from that, that you do not care for records of cases first seen at autopsy? Or, would these also help your collection?

(Dr. Callender.) I did not say, perhaps, just what I meant. I have been asking the members of the Association to send in all their cases. That has not been successful. Now I am begging them to send in those which are especially difficult and peculiar. It won't make so much work, and perhaps we will get farther that way than by trying to get them all in. I have asked you to send all of them in, including the many hundreds that some of you have, as we want them all. So I am trying to get particularly the group of cases that offer difficulties in diagnosis at biopsy, which we can follow through to the end.

(Dr. E. B. Krumbhaar, Philadelphia.) I might add that Major Callender will even treat very kindly, material that really does not belong in the lymphatic registry at all. I know this because I sent him one myself, and there was no great harm done. So, if you are in doubt, I should say to send it along. If you don't happen to remember the exact address, probably if you just send it to Major Callender, Washington, D. C., that would be sufficient. I hope you will all take some of these blanks with you, and you will see that you don't have to make a very complete report in order to satisfy his requirements, although the more complete it is, the better.

(Dr. A. S. Warthin, Ann Arbor.) I will say just a word. I am one of those who has decided views on this subject. If Dr. Callender wants a diagnosis on his case of sarcomatous Hodgkin's, I would offer it as a typical example of a tumor of this type. We have had many of them, and I feel there is no very great difficulty in the diagnosis. Being in this game for about thirty-five years, I have classed these various neoplasms on rather hard and fixed lines, trying to show their genetic relationship; and I have become convinced that Hodgkin's, the various forms of sarcomatous Hodgkin's, of reticular sarcomas of the lymph nodes, the various aleukemic and leukemic lymphoid tumors are genetically related one to another, and form a distinct class of neoplasm.

(Dr. Callender, closing.) I think I have covered Dr. Warthin's last statement. The difficulty was in the diagnosis of the case from the biopsy made in 1921. About seventeen pathologists have seen this node, and most of them, including myself, considered it a simple hyperplasia.

At the end there was no difficulty in diagnosing the case from the necropsy material. Only one man diagnosed Hodgkin's disease on the biopsy material, and I cannot see even now why he did it.

DIAGNOSTIC RELIABILITY OF FROZEN SECTIONS. William Carpenter MacCarty, Rochester, Minn.

Abstract. Frozen sections have been used routinely in the Mayo Clinic for twenty-three years, during which time 208,255 surgical specimens including 28,833 cancers have been studied and diagnosed during operations. Proof of the correctness of diagnosis is controlled readily by subsequent removal of the whole specimen and by the subsequent clinical history. All cases have been followed, and the efficiency of the method definitely established. In fact the freshness of the material and the lack of artifacts such as naturally occur in postmortem embedded material permit more efficient use of an oil immersion lens. By this means the detailed character of the cells becomes more apparent.

In conclusion it may be said: Surgeons and other clinicians know the value of frozen sections, but many professors of pathology who have been depended upon to teach surgical pathology and furnish candidates for positions in surgical pathology have failed to investigate thoroughly frozen section methods. The surgeon, being a very practical man, must of necessity build up specially trained surgical pathologists, who will be divorced unfortunately from the field of general pathology.

Pathologists, if interested at all in tissues, are missing a great opportunity to see cells as they are; they are failing to improve their understanding of their own subject. By their lack of clinical interest and the failure to utilize fresh tissues they are rapidly becoming known to be less efficient as microscopic diagnosticians.

My plea to you is that you open your eyes to a method which will startle you after a little practice. "I have never seen such beautiful cells" is the usual comment when properly made frozen sections are demonstrated to pathologists who are accustomed to see only embedded material.

AN IMPROVED MODIFICATION OF THE WARTHIN–STARRY TECHNIQUE FOR THE STAINING OF SPIROCHETA PALLIDA IN TISSUE SECTIONS. A. S. Warthin, Ann Arbor, Mich.

Abstract. Eight years experience with the Warthin–Starry staining method for the demonstration of spirocheta pallida in single sections has shown that it is the most rapid and most efficient method for the staining of spirochetes yet published in the literature. It can be used for all tissues except brain and cord. By its use spirochetes can be demonstrated in about 60 per cent of tissues containing histological lesions of syphilis. Nevertheless, it behaves capriciously with many fixations, requiring individual modifications in order to bring out the spirochetes present. Because of this fact, this laboratory has constantly aimed at the improvement of this method. With the present modification a much higher percentage of successful staining of poorly fixed tissues, even alcoholic fixation, has been obtained. It is, therefore, highly recommended for use in the case of all tissues except the central nervous system, for which we advise the use of Dieterle's method.

A COMPARISON OF METHODS OF STAINING SPIROCHETA PALLIDA IN TISSUE. R. E. Olsen (by invitation), Ann Arbor, Mich.

Abstract. Six different tissues, each containing the spirochetes of syphilis in large numbers, were stained by six methods of demonstrating the organism in single sections. Four of the six tissues were impregnated by three block methods. All the stains used were silver nitrate reductions. The methods used were:

Levaditi, Haythorn, Jahnel, Warthin–Starry (unmodified), Warthin-Starry (nitric acid-starch modification), Wright, Dieterle, Gyenes and Sternberg, and Armuzzi and Strempel. Three of the tissues used were from congenital syphilitic material, and three from acquired syphilitic cases. The results obtained show that while no method is absolutely dependable certain stains are better suited to selected tissues than others. With few exceptions, if the spirochetes can be demonstrated by the use of silver nitrate it will be best done by the improved Warthin–Starry method or that of Dieterle.

Discussion

(Dr. E. B. Krumbhaar, Philadelphia.) Dr. Warthin's and Dr. Olsen's papers are now open for discussion.

I should like to ask Dr. Warthin if opportunity has been offered by any of the methods used to get an idea of how often spirochetes may be found in adult syphilitic conditions, particularly in such cases as one would customarily consider at autopsy to be syphilitic myocarditis or aortic valvulitis.

I should also like to suggest to those who perhaps are not quite so successful as Dr. Warthin with these methods to remember before they criticize too freely the sign the Germans put up in Albert during the World War, "Nicht ärgern, nur verwundern."

(Dr. Warthin.) Do I understand you to ask me how often we have been able to demonstrate spirochetes in the myocardium?

(Dr. Krumbhaar.) What you have considered as being spirochetes in valvulitis.

(Dr. Warthin.) With the old method, about 50 per cent of the cases. With the new method, the percentage will be higher. We have not restained all the material which has been negative for spirochetes, but which shows histological lesions. We have recently had a heart sent to us from Atlanta, Georgia, a heart in advanced decomposition, alcohol fixation; and yet, with my new method, we were enabled to find spirochetes in the typical lesions. I consider that a very great victory for the method, because with my first method, I have been emphasizing that the tissue must be just as fresh as possible; that you stand no chance at all with postmortem changes, and with alcohol fixation. With this new method, by freshening up the tissue with nitric acid, and using the new silver-starch combination, we had beautiful staining of spirochetes in this heart. The new method has not been developed long enough for us to control a large number of hearts that have histological lesions in which we were not able to find the spirochetes by the old method.

(Dr. Krumbhaar.) I understand that, for instance, in the ordinary aortic valvulitis at autopsy in syphilitic organs you expect to find spirochetes in 50 per cent of the cases.

(Dr. Warthin.) In about 75 to 80 per cent with this new method. It is not a 100 per cent perfect method.

(Dr. S. R. Haythorn, Pittsburgh.) In view of the comparatively poor showing of my method as indicated in the photomicrograph just shown, I feel called upon to say a few words in its defence. I think that these methods probably stain much better in the hands of those who work with them constantly than in those who use them only occasionally. In general, my method has yielded very satisfactory results, and I have shown some very clear photomicrographs of spirochetes stained black by it. I have tried Dr. Warthin's former method, and I was not at all successful with it in many instances. We still use my method

in our laboratory, and are well satisfied with it. But I shall be very glad to try Dr. Warthin's new technique in comparison. I am very sorry that it does not work on brain tissues, because that has been one of my big problems.

(Dr. Olsen.) I am glad the personal element was emphasized. I think that is very true. However, I was careful to point out that since we had used the Warthin–Starry method so long we should be considered more expert in that than other methods. Perhaps had we used Dr. Haythorn's method as often as we had used the other, we would get better results.

(Dr. Warthin, closing.) The personal element cannot be over-emphasized because with the Warthin–Starry method we have been able to get results in tissues where other people to whom we have taught the method and shown the method have failed absolutely to get anything. It is just an example of an individual learning the tricks of this stain. These stains are tricky as they can be, because you have to vary the amount of nitric acid and the length of time nitric acid is used, just as before we had to experiment with uranium and hydrogen peroxide, by actually coaxing the tissue. It is largely a question of the hydrogen ion concentration of the tissue.

You are able to get results, but sometimes you can't explain those results. I have had several cases of umbilical cords showing very marked histological lesions, and yet with my old method we could not get a single spirochete. Just the little trick of soaking it in nitric acid changed the tissue reaction and made the spirochetes relatively more basic in the tissue so they precipitated the silver. With such temperamental characteristics on the part of the stain, the great disparity of results produced by different workers is not to be wondered at. I think the explanation of the greater number of positive results with us is simply that we learned the temperamental characters of the stain and experimented with them until we got results.

DEMONSTRATION OF RICKETTSIA IN SCROTAL SAC EXUDATE IN EUROPEAN TYPHUS GUINEA PIGS. Henry Pinkerton, Boston, Mass.

Abstract. Because of Mooser's observation of a minute intracellular diplobacillus in the scrotal sac exudate of guinea pigs reacting to Mexican typhus, attempts were made to obtain a similar picture in European typhus guinea pigs. These attempts were at first unsuccessful because observations on the scrotal sac were made at too late a stage in the course of the disease.

Mooser's observations in Mexican typhus were entirely confirmed. Mexican typhus differs from European typhus in that the scrotal reaction is much more severe, the incubation period more irregular (frequently as short as two to three days), and in the rarity of the characteristic brain lesions.

On careful observation, a slight transient scrotal reaction is occasionally seen in European typhus pigs, after intraperitoneal inoculation. If these pigs are killed on or just before the first day of fever, a gelatinous exudate is almost invariably found in the scrotal sac. Rickettsia-like organisms, similar to and probably nearly or quite identical with those described by Mooser in Mexican typhus, may be found in this exudate in European typhus pigs. There appears to be a preliminary multiplication of the virus in the scrotal sac. In the female pig, this multiplication takes place in the general peritoneal cavity, and the organism may be found in a thin layer of exudate appearing on the surface of the spleen. The exudate in the scrotal sac and the scrotal reaction occur only after intraperitoneal inoculation. This separates the disease widely from Rocky

Mountain Spotted Fever, in which the scrotal reaction is independent of the route of introduction of the virus.

Numerous brain lesions may be found in Mexican typhus if the scrotal reaction is prevented by inoculating a small amount of virus subcutaneously. In this way Mexican typhus may be made to resemble European typhus in every respect, but on subsequent passage the disease reverts to type and the scrotal reaction occurs.

The reasons for regarding the organisms as Rickettsia or as the etiological factor in the disease are: (1) their morphological resemblance to the organisms seen in the lining cells of the gut of the European typhus louse; (2) their remarkably weak affinity for dyes; (3) the failure to cultivate the organism; (4) the fact that a minute amount (0.01 cc.) of the exudate in which they are seen suffices to infect a normal pig; (5) the failure of both the microörganisms and the exudate to appear when a typhus immune pig is substituted for a normal pig.

Discussion

(Dr. R. D. Lillie, Washington.) Dr. Maxcy in the Hygienic Laboratory has recently published our observations on a similar Rickettsia, if I may use the term, in the scrotal sac of the guinea pig infected with typhus from the Southern States of the United States, which condition varies in some particulars, both from European and Mexican strains.

I have a photomicrograph showing organisms which, I think, you will consider quite similar to those that Dr. Pinkerton has just shown to us in European typhus and similar to those which Dr. Mooser also showed to us last year at Washington.

(Dr. E. Pribram, Chicago.) I should like to know how you removed the cedar oil.

(Dr. Pinkerton, closing.) Simply wash the cedar oil off with xylol and restain as before.

SYMMETRICAL OSTEOPOROSIS OF THE CRANIUM. Herbert U. Williams, Buffalo, N. Y.

Abstract. This condition is confined to the outer surface of the skull, chiefly of the parietal and frontal bones. The sutures are usually avoided. The outer table may be altogether wanting or perforated with holes up to 2 mm. in diameter. The cancellous bone is level with the surface or may protrude a little. The surface appears like pumice stone or fine sponge. It was described first by Adachi under the name of cribra parietalia. It is not related to cribra cranii, a somewhat similar condition of the inner surface of the skull. It may be related to cribra orbitalia which affects the orbital plate. Symmetrical osteoporosis may also occur on the occipital, less often on the temporals and sphenoids, very rarely on the face. There are very few reports as to the rest of the skeleton; such information as we have indicates that the long bones were rarely affected. The histological changes in sections made from the parietal bones of two cases consisted of diploë with large spaces for the marrow that opened widely on the surface; in one of them osteophytic growths of similar character protruded above the surface. Sections of ribs, from two other cases, where osteoporosis of the skull was well marked, showed the spaces of the cancellous bone to be unusually large but the outer surface was not affected and the ends of the ribs did not appear like those of rickets. All of these were in young Italian children; the soft parts were missing. Apparently an early acute stage might be followed by heal-

ing, leaving the skull permanently altered. A few cases have been seen in Indonesians. It occurred among the ancient Egyptians, but I am unable to determine how frequently. Todd informs me that he has seen it once in six hundred modern Negro skulls. If it occurs in the modern white race it must be very rare, which explains why the condition is little known among general pathologists. It was frequent among the ancient Indians of Peru; Yucatan, New Mexico and Arizona. That region is also the area in which the cultivation of Indian corn or maize was most highly developed by the Indians before the arrival of white men, and Indian corn is said to be lacking in vitamin C and probably in vitamin D. It has occurred to me that the occiput of the Indian baby commonly rested on a head board, and that may have produced a congestion of the top of the skull by impeding the outflow from the veins of the occipital region. Symmetrical osteoporosis may be allied to rickets. However the changes that we usually consider characteristic of rickets have not thus far been reported in cases of symmetrical osteoporosis. Such information as we have indicates that rickets was a rare disease among ancient and primitive peoples. Autopsies on modern Indians might well throw light on the nature and causation of symmetrical osteoporosis. The two X-ray plates that I have seen made from dried skulls showing this condition give striking pictures that might aid in the diagnosis of living cases. The X-ray shows radiating lines like bristles at the level of the outer table that are evidently produced by the new growths of bone in that situation. (Later note: I have recently learned that similar X-ray pictures have been seen a few times in certain anemias of children.)

Discussion

(Dr. A. S. Warthin, Ann Arbor.) I should like to ask Dr. Williams what criteria he uses for diagnosis that these bone changes are pathological and how he would differentiate them from acid soil erosions or from ant lesions.

(Dr. Percival Bailey, Chicago.) In the Peabody Museum at Harvard University, there is a large collection of such skulls from the abandoned Maya towns in Yucatan, and Dr. Sosman of the Brigham Hospital has made excellent radiographs of several of them.

(Dr. I. P. Lyon, Buffalo.) I should like to ask Dr. Williams if he noticed this condition among the flathead Indians, where pressure had been conspicuously applied to the skulls?

(Dr. D. P. Seecof, Denver.) I recall an autopsy on a white man from New Mexico who died of generalized lymphosarcomatosis. The X-ray pictures of the skull and the gross appearance of the calvarium, if the soft tissues had been removed, would be identical with those shown by Dr. Williams. I shall be glad to send the skull to Dr. Williams. Of course it is a single case, and may have little to do with the cases he reported, but the striking similarity of the gross and microscopic pictures made me bring up the subject of sarcoma.

INTRACRANIAL CHORDOBLASTOMA. Percival Bailey, Chicago, Ill.

Abstract. Discussion of the origin, structure, and symptomatology of intracranial chordoblastoma, with the report of two cases, one verified at necropsy, and the other at operation.

TRAUMATIC PORENCEPHALY. R. H. Jaffé, Chicago, Ill.

Abstract. The pathogenesis of porencephaly is still a matter of discussion and though numerous cases have been reported a few of the publications only deal

with the microscopic anatomy of this condition. The traumatic theory seems to be the most satisfactory one, and additional proof is yielded by the study of the brain of a woman aged 22 years, who since her earliest childhood had been suffering from epileptic fits. A large cystic defect was found in the left occipital lobe communicating with the lateral ventricle and covered by a thin membrane. This membrane was not simply formed by the leptomeningi as has been suggested in several similar cases but was composed of glia tissue with nerve fibers. Myelin sheaths could not be detected. A striking feature was the presence of many dark brown intracellular pigment granules, most of which were found to be composed of hemosiderin. By inquiry, it was learned from the relatives that the patient immediately after birth was dropped to the floor. The accident was followed by unconsciousness that lasted for several hours. The epileptic attacks started right afterwards.

Discussion

(Dr. E. R. LeCount, Chicago.) I should like to recall to Dr. Jaffé's attention, that warty papillary overgrowths of neuroglia with an arrangement of the cells suggesting rosettes were in the tissue lining the defect in one of the porencephalic brains studied by Dr. Semerak (*Arch. Neurol. & Psychiat.*, 1925, xiv, 365–383).

THE PARS INTERMEDIA OF HYPOPHYSIS IN MAN AND IN ANTHROPOID APES. Alfred P. Plaut, New York City.

Abstract. The hypophysis cerebri usually is believed to consist of three portions, namely the anterior glandular part, the pars intermedia, and the posterior nervous part. The discussion on the functions of the three different lobes is far from being closed. The activity of extracts prepared from the different lobes has been foremost among the arguments. The intermediate lobe has been held to be a "metabolism gland," its disorders leading to Froelich's disease. In 1922 the author pointed to the smallness and morphological insignificance of the pars intermedia in the adult human being, asserting that an organ which looks so rudimentary and is so variable in normal individuals can hardly have an important function. In the same year it was found that in a chimpanzee the pars intermedia was as rudimentary as in man, while the few descriptions of the hypophysis in monkeys show a pars intermedia similar to that of other mammals. In a complete series of the hypophysis of an orangoutang the pars intermedia is found even more rudimentary.

Pending investigations on other apes, monkeys and lemurs it can be stated that the hypophysis of man and apes differs essentially from that of other mammals, notably of our laboratory animals. An active principle, found in the pars intermedia of some mammal, probably is not present in the human being. Since the hypophysis plays an important rôle in growth, development and maintenance of body proportions, and in sex life this peculiarity of the hypophysis, common to man and apes, may claim anthropological and zoölogical interest in addition to its importance in endocrinology.

SARCOMA OF THE FLEXOR MUSCLES OF THE LEFT LEG. Edwin F. Hirsch, Chicago, Ill.

Abstract. A malignant tumor arising in the flexor muscles of the left leg produced extensive metastases in the lungs and lymph glands. A groin lymph gland removed several months before death contained long spindle-shaped cells with cross-striations. In the extensive secondary growths obtained for study by the postmortem examination there were cells of primitive or abortive muscle

structure with acidophilic cytoplasm, with an ectoplasm resembling a sarcolemma, with one or more vesicular nuclei, and with concentric fibrils having alternating light and dark bands or with protoplasmic prolongations having longitudinal fibrils and cross-striations like the light and dark bands of skeletal muscle. The cells in many of the metastases contained large quantities of glycogen.

The complete report will be published in the *Archives of Pathology.*

RECONSTRUCTION STUDIES ON BLOOD VESSELS OF THE SPLEEN. Ward J. MacNeal, New York City.

Abstract. Human spleen, perfused and fixed in the distended state has been used to prepare serial sections at a thickness of 5 microns. Drawings at a magnification of twenty diameters have been made on paper, and the drawing of every tenth section transferred to a wax plate 1 mm. thick for preparation of a model showing the general relations of the larger blood vessels and the follicles.

A second series of drawings at a magnification of 200 diameters has been made, and the drawings of every section transferred to 1 mm. wax plates for preparation of a model of the arterial vessels of a single lobule.

The models are not finished. However the work has progressed far enough to show that the splenic lobule can be clearly defined by the distribution of the arterial vessels, which are, in general, terminal for each lobule. Arterial communication at the periphery of the lobule is extremely rare, having been observed only twice, so far, in our preparations.

PATHOLOGY OF SICKLE CELL ANEMIA. Bernard Steinberg, Toledo, O.

(*Abstract not received.*)

ABNORMAL HEART: AORTA FROM RIGHT VENTRICLE, ABSENCE OF PULMONARY ARTERY, DEFECTIVE SEPTUM: DEATH ON THE ELEVENTH DAY. Milo K. Miller (by invitation), and M. W. Lyon, Jr., South Bend, Ind.

Abstract. Report of congenital malformation of the heart. Complete absence of pulmonary artery, the aorta arising from the right ventricle, and the left ventricle communicating with the right by a circular opening beneath the right posterior cusp of the aorta. Lungs supplied by two relatively large bronchial arteries from the descending aorta. Heart enormously hypertrophied, especially the right side. Pressure on esophagus by heart interfering with nutrition. Constant slight cyanosis with severe exacerbations increasing in progress on twelfth day, resulting in death. Hypertrophy of heart, especially of right side, shown by roentgen-ray examination.

Discussion

(Dr. Lyon.) There was no vessel at all leaving the left ventricle. The ventricle itself was really hypertrophied. It had to force the blood out against the contraction of the right ventricle at the same time, it was hypertrophied; but not so much as the right ventricle. The left auricle had four openings into it for the pulmonary veins.

(Dr. B. Halpert, Chicago.) I should like to ask whether the pulmonary artery or the veins connecting showed any anomaly at this time.

(Dr. Lyon.) The pulmonary artery was lacking, but the pulmonary veins seemed to be perfect as far as I could see. The specimen was taken out rather hurriedly, and some of the finer details were missed.

(Dr. E. B. Krumbhaar, Philadelphia.) I am sorry that Dr. Maude Abbott is on her way to Europe so that she cannot participate in this discussion. May I ask the condition of the left ventricle? Of course, there was much atrophy. Was there no sign of even a rudimentary vessel leaving?

PERSISTENT RIGHT AORTIC ARCH IN ADULTS — TWO TYPES. Aaron Arkin, Chicago, Ill.

Abstract. A new form of aortic anomaly is presented. It consists of a total persistence of the right aortic arch, together with a persistence of the left aortic arch in the form of the left subclavian artery, a short obliterated vessel and a diverticulum of the aorta. The patient presented a characteristic X-ray picture due to the position of the arch of the right-sided aorta behind the trachea and esophagus. The diagnosis was confirmed at autopsy. The heart was normal. A new X-ray picture is thus established and confirmed postmortem. Three other cases have been seen by the writer. The anomaly is of great embryologic and clinical interest.

A second type of persistence of the right aortic arch is also reported. Here the aorta rides the right bronchus, the arch remains on the right side, with the left descending aortic root behind the esophagus.

PERIARTERITIS NODOSA — HISTOLOGIC CHANGES IN EARLY AND END-STAGE. Aaron Arkin, Chicago, Ill.

Abstract. Periarteritis nodosa is a specific infectious disease probably due to a filterable virus, with an elective affinity for the arteries of the body. The organs most commonly involved are the kidneys, liver, heart, muscles, peripheral nerves, and gastro-intestinal tract.

In one acute case the writer has found as the earliest changes, periarteriolar hemorrhages due to hemorrhage per diapedesin. In other small arterioles edema with a fibrinous exudation occurred, even before leucocytic infiltration.

In another case, of a patient who had only one acute febrile attack four years before death occurred, only the histologically healed end-stage was found. Cardiac and renal insufficiency were the cause of death. At autopsy hepar lobatum, high grade coronary stenosis, contracted kidneys and myomalacia scars were found. The arterial changes were quite remarkable, with intima proliferation, extensive arterial wall destruction with aneurysms and thrombosis, periarterial granulation tissue mantles, infarction in various organs. No acute inflammatory arterial changes were found in any of the organs.

Discussion

(Dr. A. B. Wadsworth, Albany.) I am interested in the lesions that have been described and wonder whether or not there is any information regarding the bacterial etiology of the arterial changes that have developed from this condition, which appears to be an inflammatory process. These lesions resemble the lesions we find in immunized horses, especially those dying after, or killed during, immunization with the pneumococcus. Without detailed analysis, these pictures are quite similar, not only the hemorrhages of the early lesion of the practically normal vessel but also the obliterating processes in the later stages.

It might be of interest to compare these two inflammatory processes. I described some of the lesions in the *Journal of Medical Research* (*J. Med. Res.*, 1918–19, xxxix, 279), but not with particular attention to the arterial changes, although they are shown in some of the illustrations.

(Dr. E. R. LeCount, Chicago.) Perhaps Dr. Arkin is aware that an example of periarteritis nodosa that was quite well healed was recently found in the County Hospital here.

With regard to the little hemorrhages or partial zone of hemorrhage about one of the vessels in the brain, I should like to suggest simply for the thinking of all of us here that possibly such hemorrhages are caused by handling the brain after death. They are frequently found without any tissue reaction at all about them.

(Dr. E. B. Krumbhaar, Philadelphia.) Eighteen months ago I happened to be in Professor Boycott's laboratory in London while he was doing an autopsy on a somewhat similar case. I recall in it many of the symptoms that Dr. Arkin described: a septic type of fever, hematuria, I think, pains in the limbs, and then symmetrical gangrene of the feet, and death. It was noticed that some of the arteries were diffusely thickened.

When I passed through London on my return several months later, Dr. Boycott had slides which showed a very distinct acute inflammatory process involving the various coats, somewhat resembling this, but without any nodosities. There, apparently, had been no ruptured hemorrhage, no healed aneurysm. It is probable at least that that also belonged to this type and that we must think of periarteritis nodosa as a disease that may be much more than a periarteritis and often not nodose.

Dr. Arkin is probably also aware of an infectious type of this disease that is supposed to occur in horses, which perhaps would be some answer to Dr. Wadsworth's query.

(Dr. Arkin, closing.) In answer to the question about hypertension, I want to say that in five cases I have seen, hypertension has been present only in those cases where very marked renal changes were shown. There may be a nephritis, or only extensive infarction, or both. In cases where there were no marked renal changes, there was, as a rule, no hypertension. In fact, the blood pressure was low.

In answer to the question about changes similar to these in animals, I want to emphasize that these changes were limited strictly to the arteries. There were no changes in the veins. The disease has been found in lower animals. It has been described in the calf, swine, dog, deer. It sometimes occurs in epidemic form. The histological changes resemble those in man.

I am glad Dr. LeCount called attention to the possibility of trauma as the case of periarteriolar hemorrhages. I simply showed it because it was found in the same organs where the other changes were present. Whether or not these are due to periarteritis nodosa, I can't state definitely. The brain was removed as carefully as possible.

The healed case is striking because the changes were all healed. In the cases of periarteritis nodosa that have been reported, inflammatory changes were also present. But to find a case such as this which was completely healed histologically, is a rare thing and I have not seen it before.

THE INVOLVEMENT OF MEDIUM-SIZED ARTERIES IN SYPHILITIC AORTITIS. Otto Saphir, Cleveland, O.

Abstract. Among 50 cases of syphilitic aortitis, the innominate artery showed syphilitic changes in 33, the carotid in 29, the superior mesenteric in 10, the inferior mesenteric in 3, the common iliac in 10, and the femoral artery in 7 cases. The subclavian artery was examined in 29 cases, 15 of which showed syphilis.

The syphilitic lesions were characterized by endarteritis of the vasa vasorum, and perivascular infiltration of lymphocytes in the adventitia. The media of the elastic type of arteries showed an interruption of the continuity of the elastic fibers and fibrotic areas combined with circumscribed lymphocytic infiltrations. The media of the muscular type of arteries only rarely showed changes.

In the intima, circumscribed button-like areas of fibrosis, without degenerative changes, were found very frequently. These are not specific but are chronic inflammatory in nature and might be associated with any type of pathological lesion in the adventitia.

Discussion

(Dr. B. J. Clawson, Minneapolis.) I should like to ask Dr. Saphir if he has had any experience with a coronary artery. I have examined coronary arteries in many cases of syphilitic aortitis and have not been able to find syphilitic lesions in them.

(Dr. A. Plaut, New York City.) What stain do you use? The orcein stain?

(Dr. Saphir, closing.) I have examined the coronary arteries in about 25 cases and I have never seen definite changes in the coronary artery directly attributable to syphilis. Spirochetes have never been seen in the aorta or in the vessels of the branches of the aorta.

The stains we use have been important. Warthin's stains and our stains, I think, have been instrumental in our success. The sections are placed in an alcoholic solution and then in the incubator for two hours; we then differentiate them with a 0.5 per cent solution of hydrochloric acid.

STUDIES ON THE PATHOGENESIS OF INFECTIOUS ENDOCARDITIS. K. Semsroth and Robert Koch (by invitation), Pittsburgh, Pa.

Abstract. 1. In paratyphoid or staphylococcus infected guinea pigs, hypersensitive to horse serum, there developed a nodular mononuclear endocardial proliferation with regularity, while non-sensitized controls only occasionally showed such reaction. Desensitization before infection practically prevents the reaction. Desensitization after the infection does not alter the result.

2. An allergic state was induced in rabbits by repeated intravenous *casein* injections. A hyperergic skin reaction was obtained after five to eight casein injections.

Subsequent infection with staphylococcus aureus led to a definite *endocarditis* in six out of eight animals.

ATTEMPTS TO REPRODUCE RHEUMATIC DISEASE IN ANIMALS. Louis Gross, Leo Loewe, and (by invitation) Benjamin Eliasoph, New York City.

Abstract. One hundred and two animals representing seven species were injected over a period of from one week to one year with twenty-one strains of streptococcus gamma, most of which were obtained from blood cultures of rheumatic disease cases. There was also injected a number of materials such as whole blood, serum, plasma, pericardial fluid, hydrocele fluid, filtrates of subcutaneous nodules, tonsils, lymph nodes, nasopharyngeal washings, etc. The majority of the material was obtained from cases where autopsy, biopsy (subcutaneous nodules), electrocardiograms, roentgenograms, and clinical findings made the diagnosis of rheumatic disease beyond question. Injections were made through a variety of routes, such as intracardially, intravenously, into the tonsils, nasopharyngeal mucous membrane, lungs, joints, peritoneal cavity, etc. In order to lower the resistance of the animals, a variety of procedures was

adopted, *viz.*, injections with Trypan blue, poisoning heart muscle with Digitan, subcutaneous injection of infected agar mass, thrombosis of superficial veins with subsequent infection of thrombus, etc. A number of lesions, including inflammatory myocardial foci, vegetative endocarditis, pericarditis and arthritis, were found. Criteria are discussed whose fulfillment are necessary in order to establish the experimental reproduction of rheumatic disease. According to these criteria, we have failed to reproduce the disease in animals.

Discussion

(Dr. B. J. Clawson, Minneapolis.) I should like to know what is meant by the typical Aschoff nodule.

(Dr. Gross.) Before I answer Dr. Clawson's question, I should like to take a moment or two in order to elaborate on several of the points which Dr. Loewe brought out.

I want to make it clear that we are not attempting to criticize caustically the past work in this field. Indeed, when we commenced our investigations several years ago, we were very hopeful of reproducing this disease by means of streptococci. However, in the light of our experience we must conclude that we have not reproduced rheumatic fever, nor do we believe that other workers have had any greater measure of success. On the other hand, we do not desire to leave the impression that we dogmatically deny the possibility that the streptococcus may be the etiological agent.

There are at present, broadly considered, three avenues of approach in the investigation of this disease: the bacteriological, the immunological and the anatomical. The bacteriological method has concerned itself with attempts to obtain organisms from rheumatic fever patients, principally from the blood, and to show by their relative frequency of occurrence, as well as by their serological identity, that they are responsible for the disease. Thus for example, Dr. Clawson found that seven of his ten streptococcus viridans strains fall into one serological group, and Dr. Kreidler found that 107 strains of his streptococcus and cardioarthritidis strains also fall into one group. I believe, however, that neither of these investigators carried out absorption tests to prove that they were not dealing with group agglutination. It seems that, as a whole, the bacteriological methods have led to no definite conclusions.

As examples of the immunological approach, one can briefly cite the work of Birkhaug on skin reactions, also the work of Dr. Swift and his collaborators. Dr. Birkhaug's work is very interesting, but it seems to me that all that we can conclude from his results is the fact that rheumatic fever patients show a marked skin susceptibility to certain streptococcus products. Dr. Swift and his co-workers have shown that the reactions in the tissues may depend on their immunological state. Attempts to interpret these interesting observations in terms of rheumatic fever, however, bring up many difficulties, not the least of which is the infrequent coincidental occurrence in the same heart of both subacute bacterial endocarditis and acute rheumatic disease, as evidenced by the presence of Aschoff bodies.

The therapeutic approach which properly falls under the immunological group presents obvious difficulties, for here one must eliminate non-specific protein effects and one must have clear-cut statistics on a large scale. An interesting example of the conflicting views presented by such work appears in the report of the Bath Conference on rheumatism, where Dr. Small presented his therapeutic results following the use of streptococcus cardioarthritidis serum

and vaccine, and Bertrand claimed even better results with his Achlame bacillus vaccine.

There is, therefore, left, as Dr. Loewe stated, the anatomical approach. He has shown you why our results lead us to the conclusion that the disease has not as yet been reproduced.

In answer to Dr. Clawson's question, I must say that, at any rate in our laboratory, we have the feeling that if there is any doubt in our minds as to whether a certain collection of cells is or is not an Aschoff body, we are not justified in calling it an Aschoff body. How much more critical, therefore, must we be in the case of experimental animals? Here the burden of the proof is obviously up to the experimenter.

(Dr. Clawson, closing.) I should like to correct Dr. Gross about my making claim to the specificity of the group of streptococci. I think if he refers to my paper he will find that I emphasized the fact that the streptococci isolated from patients with acute rheumatic fever did not represent a specific group. They were not a specific strain.

A Comparison of Rheumatic and Experimental Streptococcic Inflammations. Benjamin J. Clawson, Minneapolis, Minn.

Abstract. Rheumatic inflammation may exist as nodules or as irregular groups of large mononuclear and multinucleated cells, lymphocytes, plasma cells, eosinophils and a varying number of polymorphonuclear leucocytes.

The rheumatic nodule, called the Aschoff body when seen in the myocardium, is a small, fairly well localized area of inflammation, mostly polyblastic in character, but at times chiefly or entirely exudative. It varies greatly in size and shape. It may consist of but a few cells or be large enough to be seen with the naked eye. It may take the form of a rosette or become more or less fan-shaped or fusiform. The inflammation may extend out irregularly into the interstitial tissues and at times become so extensive as to form a diffuse myocarditis.

In a relatively high percentage of rabbits injected in the left ventricular cavity with agglutinated streptococci, nodular inflammatory areas, with a cellular reaction similar to that found in the human Aschoff bodies, may be produced in the myocardium.

The subcutaneous rheumatic nodule has a cellular reaction similar to that found in the Aschoff nodules in the heart. By injecting streptococci in small amounts into the subcutaneous tissues of rabbits, lesions can be produced which are morphologically similar to the nodules found in the subcutaneous tissues or the myocardium in cases of acute rheumatic fever.

The arterial lesions in cases of acute rheumatic fever consist of a swelling of the endothelium which becomes basophilic and may be raised from the basement membrane by a coagulated exudate. There is cellular necrosis in all parts of the vessel wall. Exterior to the necrotic wall there is a cellular exudate, many cells of which are polymorphonuclear leucocytes showing various stages of necrosis. In this area there are also plasma cells, and mononuclear and multinucleated cells similar to those found in rheumatic inflammation in other parts of the body.

Similar lesions may be produced in monkeys and rabbits by injecting streptococci intravenously and subcutaneously.

The similarity of the inflammatory reaction commonly found in cases of acute rheumatic fever to the reaction produced experimentally by injecting strepto-

cocci further suggests a causal relation between the streptococci and rheumatic fever.

Discussion

(Dr. K. Semsroth, Pittsburgh.) I should like to emphasize what Dr. Bell has said. While from a clinical and bacteriological standpoint it may be possible to differentiate between various forms of endocarditis, such a clear differentiation is *not* possible on the basis of the morphological picture of the heart lesions. "Rheumatic" myocardial nodules have been observed more than once in cases of non-rheumatic heart lesions. In our experience, intravenous treatment of rabbits with casein and subsequent staphylococcus infection was sometimes followed by the development of large mononuclear subepicardial nodules which looked much like several of the pictures which Dr. Clawson has shown.

(Dr. E. T. Bell, Minneapolis.) I should like to raise a question for those who think that rheumatic endocarditis and bacterial endocarditis are two distinct diseases. If you define rheumatic endocarditis anatomically as firm vegetations without thrombi and bacterial endocarditis as vegetations with thrombosis, you will find that half the cases of endocarditis are of mixed type, since both types of vegetations are present. Rheumatic vegetations are found in half the cases of clinical bacterial endocarditis. Are we going to argue that over half of the cases of rheumatic fever are merely complicated by streptococcus infection?

You will also find that the clinician will change his diagnosis after he watches the case a while. If the patient gets well it is rheumatic endocarditis. If he dies, it is bacterial endocarditis.

(Dr. L. Gross, New York City.) I should like to ask Dr. Clawson whether, if he were presented with slides from a human rheumatic heart and he did not know that the sections were from human material, he would under any circumstances be willing to commit himself as to whether the lesion is one of rheumatic fever, and, if so, what picture he has in mind when he so commits himself. Furthermore, how would he differentiate the Aschoff body from the type of multinucleated and large mononuclear collections frequently found in the rabbit's myocardium.

I should like to ask Dr. Clawson whether he has attempted to reproduce subcutaneous nodules by means of organisms other than streptococci.

Just one word about Dr. Bell's question. I was not quite clear as to whether Dr. Bell meant to indicate that he has found typical fresh rheumatic verrucae side by side with typical bacterial vegetations in so large a proportion of cases. I don't mean of course the typical healed verrucae which obviously represent previous rheumatism. I must say that our experience has been entirely different in this respect. We have found typical fresh rheumatic verrucae and Aschoff bodies coexistent with the typical subacute bacterial endocarditis, but this has been a very infrequent observation.

(Dr. Clawson, closing.) In regard to the question if a slide were placed before me from the heart of a human endocarditis as to whether I would select it as that of a human, I should say no. I do not think these nodules are specific for rheumatic infection. They are more common in rheumatic infection than in subacute bacterial endocarditis.

I am glad Dr. Gross brought that out because I was going to refer to the article describing a case of meningococcic endocarditis in which there were nodules which Dr. Rhoads and Dr. Mallory passed as Aschoff nodules.

Theodore Farr has long stressed the fact that he has found the Aschoff nodule in myocardium in cases of scarlet fever. Dr. Warthin has pointed out the simi-

larity between syphilitic infections and this type of infection. In going over the various cases of syphilitic aortitis in 8 per cent I found in the myocardium, near to the aortic ring, nodules which I would call Aschoff nodules.

In relation to the definition of Aschoff nodules, I would say: they are localized polyblastic areas of inflammation, but at times there is exudation to the extent of being almost an abscess, and I think we can find all stages from what is called the typical Aschoff nodule right down through to the abscess. Aschoff himself emphasized the fact that polymorphonuclear leucocytes were conspicuous in some of these nodules.

I want to emphasize the question of both lesions occurring in the same heart. We have had cases checked clinically where the clinician was not certain whether he had an acute rheumatic endocarditis or a bacterial endocarditis. Dr. Bell said it occurs in one-half of the cases. That is very conservative. I think in our paper we say three-fourths of the cases of subacute bacterial endocarditis show both types of lesions.

SKIN REACTIONS AMONG RHEUMATICS TO STREPTOCOCCAL PRODUCTS. Konrad E. Birkhaug (by invitation), Rochester, N. Y.

Abstract. During May to September, 1928, a series of 594 persons, among whom were 118 active and inactive cases of rheumatic fever, were studied for skin hypersensitiveness to streptococcal products. These cases were scattered in clinics in London and Birmingham (England), Bergen (Norway), Copenhagen (Denmark) and Hamburg (Germany). Altogether 3114 skin tests were made with one or several comparable concentrations of filtrates, autolysates and vaccines of hemolytic, viridans and indifferent streptococci, isolated from rheumatic fever sources. A marked universal hypersensitiveness was found to exist among active and inactive cases of rheumatic fever, carditis, or chorea, to filtrates, autolysates and vaccines produced by the viridans and indifferent streptococci, and moderately to the autolysates of the hemolytic streptococcus. Inactive cases of rheumatic infection react less to the non-hemolytic streptococcal products than active cases of rheumatic fever, carditis, or chorea. Filtrate boiled for four hours produces exaggerated skin lesions in active cases of rheumatic fever almost as frequently as the unheated filtrate, while reactions with the boiled filtrate are almost nil in inactive rheumatic infection. Salicylates exert no effect upon the capacity of skin to react hyperergically with the streptococcal products in active or inactive rheumatic infection. More than 70 per cent of rheumatic fever, carditis or chorea react positively to the non-hemolytic streptococcal products, while about 25 per cent of non-rheumatics give positive reactions. Cases of chronic infectious arthritides react to the viridans and indifferent streptococcal products in almost the same proportion as inactive cases of acute rheumatic infection, while degenerative chronic types of arthritides behave like non-rheumatic controls. It appears that a common allergenic factor is present excessively in the viridans and indifferent streptococcic cells and moderately in the hemolytic streptococcus, which reacts universally when injected intradermally in persons stigmatized by rheumatic fever. No evidence is available of a strictly specific allergenic agent. No parallel reactions occur in rheumatic infections with the Dick or Schick tests.

Discussion

(Dr. R. R. Mellon, Pittsburgh.) I should like to ask Dr. Birkhaug whether any of these patients have been tested out with organisms other than strepto-

cocci. I ask this question for two reasons: First, because I have had occasion from time to time to observe positive skin tests of rheumatics with certain other organisms to which they appeared to be abnormally sensitive, and partly because we were impressed with this non-specific or allergic factor in my work with Drs. Koch and Semsroth, particularly as evidenced by the non-specific action of casein in our experimental cardiac localizations.

(Dr. Birkhaug, closing.) It was desirable to perform a great many more skin tests on these children and adults with heterogenous bacterial substances. But to perform nine skin tests, particularly on a child, is a pretty gruesome ordeal, especially when done in the out-patient clinic. That is the sole reason for confining these studies to the three varieties of streptococci. Further studies, however, are contemplated wherein products of other organisms will be employed.

DOES SPLENECTOMY INFLUENCE THE LEUCOPENIA INDUCED BY CERTAIN FOREIGN SUBSTANCES? Isolde T. Zeckwer, Philadelphia, Pa.

Abstract. Doan, Zerfas, Warren, and Ames (*J. Exper. Med.*, 1928, xlvii, 403) have recently reported interesting experiments in which they found that sodium nucleinate injected intravenously in normal rabbits resulted in a marked leucopenia in the peripheral blood, which lasted for a number of hours, when it was succeeded by a leucocytosis. They believe that during the peripheral leucopenia, the leucocytes have collected exclusively in the spleen, as indicated by leucocyte counts made on the blood from the viscera, and by their finding that after splenectomy, sodium nucleinate resulted in no period of leucopenia, and that leucocytosis then began within a period of time one-half to one-sixth of that required in animals in which the spleen was intact.

It seemed of importance to determine whether these findings of the effect of splenectomy are of universal application to the reaction to foreign substances in general or apply only to the reaction to the specific substance sodium nucleinate. If the spleen has such a function of storing and then discharging leucocytes, it would be of as great significance as the mobilization of red cells by the spleen from its reserve, in conditions of great need, as demonstrated by Barcroft.

To determine this point, *B. coli* vaccine was injected intravenously into rabbits, and leucocyte counts were made on the ear blood at frequent intervals after injection. The leucocytes fell abruptly and remained at a low level for several hours. The curves thus obtained were compared with curves obtained after vaccine in the same animals at different times after splenectomy. It was found that splenectomy had no effect on the degree of leucopenia, or on the time of rise of leucocytes after the leucopenic period.

These results necessitated a reinvestigation of the effects of splenectomy on the leucocyte changes following the injection of sodium nucleinate. Doan and his associates used sodium nucleinate in doses of 1 gm. per rabbit. When this dosage was used in the present experiments, the animals showed marked circulatory depression. It was found that a much smaller dose, 0.1 gm. per rabbit, was just as effective in producing leucopenia and resulted in no obvious vascular changes, so that the animals could be bled readily, and this dosage was well tolerated, so that injections could be repeated frequently for comparison. It was found that in every animal used, splenectomy did not prevent the occurrence of a marked leucopenia, in doses of 0.1, 0.5, and 1 gm. The time of rise in leucocytes varied greatly on different occasions in the same animal before splenectomy, and there was no evidence that splenectomy resulted in a more

rapid return rise of the leucocytes than the same animals had shown before splenectomy.

Blood pressure tracings were made in several animals. With 1 gm. doses the blood pressure fell to a low level on injection of sodium nucleinate, and this low level was maintained for a long time. With 0.1 gm. doses there was a transient fall in blood pressure, but a rapid return to normal, and the leucopenia was maintained while the blood pressure remained at the normal level.

It is concluded that the leucopenia following injections of sodium nucleinate, or bacterial vaccines, is not of splenic origin, and that there is no evidence in these experiments that the spleen exerts any regulatory function over the distribution of leucocytes within the body.

EFFECT OF REPEATED EXPOSURE TO HIGH TEMPERATURE ON THE LEUCOCYTE COUNT IN MICE. O. R. Caillet (by invitation) and J. P. Simonds, Chicago, Ill.

Abstract. Murphy and Sturm showed that a definite leucocyte curve could be produced by subjecting mice, on a single occasion, to dry heat. Nakahara, in the same series of animals, demonstrated changes in the lymphoid tissue which he considered definitely associated with the blood picture produced by the dry heating. It has been the object of this study to observe the effect upon the blood and lymphoid tissue of repeatedly submitting mice to dry heat in the manner described by the workers cited above. Groups of mice were heated for five minutes at a temperature of 60° C at intervals of ten days over a period of two hundred and ten days. During the first fifty days there was a marked fall in the white cell count after each heating, accompanied by cellular necrosis in the spleen and followed by the appearance of many mitotic figures in the germinal centers, and a rapid rise in the white cells in the blood stream. During this initial period there was a stair-step rise in the white cells principally lymphocytes up to 25,000 per cmm. During the remainder of the period of heating there was a gradual fall to and below the normal leucocyte count. The fluctuation following each heating was very small. Sections of the spleen at this time showed a marked fibrosis with loss of germinal centers.

Discussion

(Dr. W. J. MacNeal, New York City.) The paper is before you for discussion.

I should like to ask whether there were any observations upon the internal temperature of the mice immediately following the exposure to heat.

(Dr. Caillet.) In our observations we did not notice the internal temperature. However, Murphy noticed an internal temperature rise of about one to two or three degrees centigrade immediately following the heating.

(Dr. MacNeal.) Maximum about 40° to 42°?

(Dr. Caillet, closing.) Not so much, I should say.

COAGULABILITY OF THE BLOOD DURING MECHANICAL OBSTRUCTION OF THE HEPATIC VEINS. W. W. Brandes (by invitation) and J. P. Simonds, Chicago, Ill.

Abstract. The changes that occur after constriction of the hepatic veins by a method previously described by us are: (1) a precipitate fall in arterial blood pressure; (2) a decrease followed by a slower increase in blood concentration; (3) a decrease in sugar content of the blood; and (4) a decrease in coagulation

time. After release of the constriction of the hepatic veins the blood pressure rises sharply, a temporary hyperglycemia is produced and the coagulation time is increased. During the constriction the liver shows marked congestion, and pressure in the portal vein is increased.

Various studies have been attempted in an endeavor to explain the decrease in coagulation time, but as yet no definite conclusions have been reached. Since a normal dog may show variations in coagulation time, it is difficult to interpret the results obtained. That this decrease in coagulation time after constriction is not due to the anesthetic and trauma is, we believe, shown by the fact that it occurred also in dogs which had been kept under ether anesthesia and subjected to trauma for varying periods of time before the hepatic veins were constricted. The average coagulation time in fifteen dogs before constriction was three minutes, while within five minutes after constricting the hepatic veins it was one minute fifteen seconds.

Changes in platelet counts are not sufficiently marked to account for the change in coagulation. There seems to be a general tendency to a slight decrease throughout the experiment, but there is no such decrease as occurs in peptone shock.

Fibrinogen determinations were not constant. But there appears to be a slight tendency to a decrease in fibrinogen during constriction of the hepatic veins.

Blood calcium shows but little variation during the period of constriction. In these experiments the hydrogen ion content of the plasma showed a moderate increase.

From the above it is difficult to explain the cause of the change in blood coagulability. Shutting the liver out of the circulation may prevent heparin from entering the circulation. Also in course of the changes in the concentration of the circulating blood, constituents which hasten coagulation may enter the circulation with the tissue fluids. After hemorrhage we know that the coagulability of the blood increases. Furthermore dilution of the blood may be a factor in this decrease of coagulation time.

Discussion

(Dr. Simonds.) There is one thing that might be added to what Dr. Brandes has said. It is very well known that in peptone shock there is a marked reduction in number, and sometimes an almost complete disappearance, of blood platelets from the peripheral blood. A considerable number of counts of blood platelets during constriction of the hepatic veins showed that the variations were entirely within the possibility of error by the method used.

THE EFFECT OF UNILATERAL NEPHRECTOMY ON THE TOTAL NUMBER OF FUNCTIONING GLOMERULI IN THE RABBIT. Robert A. Moore and Gregory F. Lukianoff (by invitation), Cleveland, O.

Abstract. Using the method of Hayman and Starr, the total number of open glomeruli in the kidney of the rabbit has been determined by an intravital injection of Janus Green B. The normal number of open glomeruli was found to vary from 44 to 75 per cent, with an average of 63 per cent. For one to ten days following unilateral nephrectomy, the total number of open glomeruli was found to vary from 90 to 100 per cent. Control animals subjected to a sham operation failed to show any variation from the normal. We believe that the immediate compensation for the loss of one kidney is accomplished by this increase in the number of open glomeruli.

Discussion

(Dr. E. T. Bell, Minneapolis.) I should like to ask Dr. Moore what percentage of the total capillary circulation is in use in a normal resting kidney. In his estimation he showed about 63 per cent on the average of the glomeruli in action but only parts of many glomeruli were open.

(Dr. Moore, closing.) To answer Dr. Bell's question would involve the question of the intraglomerular circulation. I have no quantitative studies, but it is my impression that approximately one-third of the capillary beds of the 63 per cent of open glomeruli are in circulation. The other two-thirds are out if we judge by this method of staining.

THE PRODUCTION OF ALLERGIC INFLAMMATION IN THE KIDNEYS. Opal E. Hepler (by invitation) and J. P. Simonds, Chicago, Ill.

Abstract. A local reaction similar to the Arthus phenomenon has been produced in the mucosa of the stomach (Schapiro and Ivy) and in the lungs (Friedberger and Opie) of animals previously sensitized to the protein injected. We have studied the local reaction in the kidneys of sensitized rabbits following the injection of egg albumin and horse serum directly into the left kidney. From one to eight intrarenal injections were given under aseptic precautions at five-day intervals, with and without a preliminary series of subcutaneous injections. The animals were killed from twenty-four hours to one month after the last injection. It was found that the injection usually produced local hemorrhage. When the antigen was injected into the cortex of the kidney, necrosis involving especially the tubular epithelium, but also, to a less extent, the glomeruli and connective tissue, occurred. This was accompanied by more or less edema and leucocytic infiltration of the area of necrosis. Inasmuch as these changes did not follow a single injection of foreign protein into the kidney of a non-sensitized animal, we believe that the changes described were due to the action of the antigen upon the locally sensitized renal tissues.

Discussion

(Dr. E. R. Long, Chicago.) This is of great interest to me in connection with results that I reported before this Society last year. I found that in tuberculous animals, when the specific product to which they were sensitive (tuberculin protein) was introduced in the kidney, distinct changes took place. Those were of more definitely inflammatory character than these which have just been reported. In only one instance was there a change which was rather similar to those just described. This was in an animal previously normal which was simply injected with a tuberculin protein. On reinjection with the same material two weeks later, a considerable amount of necrosis occurred, which I interpreted as an Arthus' phenomenon. The necrosis seemed to me to be all out of proportion to any vascular damage that could be seen in the sections. This, I think, is quite in harmony with what you have reported. Yesterday, Dr. Lewis and Dr. Seibert reported experiments on this same protein, indicating that it elicits antibody production.

(Dr. E. Ecker, Cleveland.) I should like to ask Dr. Hepler what reactions occurred when she introduced the needle alone into a kidney.

(Dr. Hepler, closing.) We injected four rabbits in all and checked them immediately, one-half hour, one hour and two hours after we injected them. All we observed was a hemorrhage, no necrosis.

THE FINER STRUCTURAL CHANGES IN THE GLOMERULI IN GLOMERULONEPHRITIS. Leone McGregor (by invitation), Minneapolis, Minn.

Abstract. A histological study of the changes in the glomeruli in glomerulonephritis has been made using Mallory's aniline blue (Heidenhain azo-carmine modification). This stain brings out the glomerular basement membrane distinctly, thus separating the endothelial from the epithelial cells.

The changes observed in the earliest stages of glomerulonephritis were as follows: (1) a marked accumulation of mononuclear and a few polymorphonuclear leucocytes within the capillary loops; (2) the appearance of delicate blue-staining hyaline fibers between the leucocytes; (3) a slight increase in endothelial cells; (4) a proliferation and often a degeneration of the glomerular epithelium; (5) a proliferation of the capsular epithelium with crescent formation; (6) the presence of hyaline blue-staining fibers between these capsular epithelial cells.

In later stages of the disease the intracapillary hyaline fibers form a definite meshwork enclosing the leucocytes and a few endothelial cells. The meshwork becomes more dense until the lumen of the capillary is almost filled with a mass of hyalin containing some cells. The blood circulation continues between the basement membrane of the loop and the periphery of the hyaline mass. Finally the mass completely occludes the capillary. Adjacent loops fuse with one another and with the capsule to produce a hyaline glomerulus.

This process is typical of all cases which are clinically glomerulonephritis.

Preliminary studies on hypertension kidneys show a different glomerular lesion consisting of an irregular thickening of the basement membrane. Intracapillary hyaline fiber formation is seen only in those hypertensives who have died of uremia and then in only a few glomeruli.

Examination of amyloid kidneys has confirmed Ohmori's observation that the amyloid is deposited inside the glomerular basement membrane.

Discussion

(Dr. E. T. Bell, Minneapolis.) I would like to say that these lantern slides do not show as well as the drawings. The lantern slides were made from colored drawings, and the membrane does not come out as well as it should. This is really an excellent stain for studying the finer changes in glomeruli.

The basement membrane is brought out sharply and with the aniline blue stain you are able to tell what is inside and what is outside of the capillary.

The hyaline fibers which Dr. McGregor has not been able to identify chemically seem to be typical of glomerulonephritis.

LIPOID NEPHROSIS. E. T. Bell, Minneapolis, Minn.

Abstract. A survey of recent literature shows a tendency to use "lipoid nephrosis" in a broad way to include all cases of nephritis with edema, albuminuria, normal blood pressure and normal blood nitrogen. This clinical definition includes many cases that are anatomically acute or mild chronic glomerulonephritis.

All the clinical phenomena of lipoid nephrosis, *viz.*, albuminuria, edema, normal blood pressure, hypercholesterolemia, decrease of the plasma proteins with reversal of the albumin-globulin ratio and normal blood nitrogen, may occur usually in a less pronounced form in glomerulonephritis.

There are many transition cases between lipoid nephrosis and glomerulonephritis, *viz.*, those that fit the definition of lipoid nephrosis except for the pres-

ence of hypertension or moderate nitrogen retention. These are called the mixed type or nephritis with a nephrotic *Einschlag*. From the clinical standpoint the two diseases cannot be sharply separated.

A histological study has been made of ten cases of large, fatty kidneys, without tubular atrophy, that were clinically nephritis. Four of these are classed as pure lipoid nephrosis, three as nephritis with nephrotic *Einschlag* (mixed type) and three as of uncertain type because of lack of clinical data.

The glomeruli are not normal in any instance, but the lesions are not of uniform type.

In pure lipoid nephrosis there is an uneven thickening of the basement membrane of the glomerular capillaries, and there may be many mononuclear leucocytes in some of them.

In the mixed type (nephritis with nephrotic *Einschlag*) there is a marked thickening of the basement membrane. In one instance the capillaries were compressed by enlarged epithelial cells. In two instances there were many leucocytes in the capillaries, and a few areas in the glomeruli showed the changes characteristic of typical clinical glomerulonephritis, *i. e.*, capillaries filled with leucocytes and a network of hyaline fibers.

Three large non-contracted kidneys were studied in which the data were insufficient to determine whether they should be classed as lipoid nephrosis. Two of these showed extreme thickening of the basement membrane. The third showed an enormous accumulation of fat in the tubules and glomeruli, large glomerular epithelial cells with hyaline and fatty degeneration, and leucocytes and hyaline fibers within the capillaries as in glomerulonephritis.

Lipoid nephrosis is to be regarded as a form of glomerulonephritis in which the glomeruli are damaged but their capillaries are only partially obstructed so that they continue to function and tubular atrophy does not occur.

Discussion

(Dr. E. R. Long, Chicago.) It is quite astonishing what changes can be brought out by the staining methods of Drs. McGregor and Bell. Many of those glomeruli appear to be normal until subjected to the special new stain. I should like to ask if they have any idea of the age of those lesions characterized by the thickening of the basement membrane shown by this technique.

(Dr. W. J. MacNeal, New York City.) I should like to ask Dr. Bell whether in his cases of lipoid nephrosis he has examined the other organs, as, for example the liver, for lipoid-containing cells; whether perhaps we may not be dealing with a much more widespread process than that merely involving the kidney.

(Dr. H. G. Wells, Chicago.) I should like to add for a little expression of satisfaction that I have been looking all my life for pure, chronic lipoid nephrosis in the adult. At autopsy I have had a number of cases in which the clinical picture was absolutely classical and the gross picture was absolutely classical of chronic lipoid nephrosis, and then, to my horror, I found typical glomerular changes of subacute or chronic glomerulonephritis.

(Dr. Bell, closing.) I do not know the significance of the hypercholesterolemia. We have no knowledge concerning that at all.

I think lipoid nephrosis is a renal disease because it shows many transitions to glomerulonephritis. Hypercholesterolemia is often pronounced in typical cases of glomerulonephritis.

There is a widespread opinion that lipoid nephrosis is a metabolic disorder,

but all the symptoms and laboratory findings characteristic of lipoid nephrosis may be found in glomerulonephritis, which is surely a renal disease.

In reply to Dr. Long's question, the case that showed the most striking hyaline changes in the basement membrane was of fifteen years duration. The patient had had edema for fifteen years. There never was renal insufficiency nor cardiac hypertrophy.

We have no accurate information concerning the duration of the other cases. The best case we had was of four weeks duration.

I have not studied the changes in the other organs as extensively as I should in order to reply fully to Dr. MacNeal. In the cases which I have studied I did not see any fatty changes in the spleen or in the liver, and there is no similarity to Niemann's disease.

CALCIFICATION OF THE KIDNEYS IN BISMUTH POISONING. C. V. Weller, Ann Arbor, Mich.

Abstract. In bismuth poisoning in man, calcification of the damaged renal tubular epithelium is found, comparable to that described for experimental bismuth nephritis in lower animals. The chief deposits of lime salts are in the straight tubules of the medullary rays and in that portion of the ascending limb of Henle which returns to pass near the glomerulus of origin. While bearing a general resemblance to the lesions produced by mercuric salts, there are minor differences which, if they are found to be constant upon further observation, may assist in differentiating the two conditions.

PATHOLOGICAL EFFECTS FOLLOWING EXCESS OF IRRADIATED ERGOSTEROL. Harry Goldblatt, and (by invitation) A. T. Shohl, Cleveland, O.

(*Abstract not received.*)

MULTINUCLEATE CELLS IN THE SEMINIFEROUS TUBULES OF THE TESTIS. David P. Seecof, Denver, Colo.

Abstract. Multinucleated or so-called "giant" cells were present in practically every seminiferous tubule of the testes of a man 42 years of age dying of septicemia three days after the onset of an acute sore throat. The "giant" cells were formed chiefly from spermatids by a process of fusion. A few similar "giant" cells and others resulting from a fusion of spermatocytes and spermatogonia were found in the testes of some individuals of all ages dying of various acute and chronic diseases. A specific etiological factor for the lesion could not be determined. The findings were in accord with those of previous writers on the subject as regards the occurrence and pathogenesis of this lesion, and a brief summary of the literature is given.

CYSTITIS EMPHYSEMATOSA; REPORT OF CASES IN MEN. Ralph G. Mills, Rochester, Minn.

Abstract. Cystitis emphysematosa, judging from the literature, is a rare condition, having been reported only eleven times by six observers. A similar condition has been seen in animals, and in these also has been confined to the female sex. The discussions have been intermingled with reports of *colpohyperplasia cystica*, and with *pneumatoides cystica intestinorum* in a very confusing manner. Studies involving the histology as well as bacteriology and chemical

examination of the gas content of the vesicles have been made on all three conditions and applied indiscriminately to the others. Opinions have varied as to whether cystitis emphysematosa is a postmortem invasion by some gas-forming organism, or whether it really exists during life. There are no reports indicating *intra vitam* diagnosis.

This study is part of a series of articles discussing various phases of the subject, and attempting to establish this condition on an independent basis, free from inference or comparison with the others mentioned. It disposes of the presumed sex-selective nature of the lesion, and tends to show that it develops during life. It indicates that a real cystitis is a part of the process, and that the vesicles are not cysts in the strict sense, being devoid of a true lining of epithelial or endothelial cells, or their derivatives. The designation "cystitis emphysematosa" apparently conveys the correct interpretation.

Discussion

(Dr. Hueper, Chicago.) I reported one case several years ago; I think that the etiology of this condition can be clarified only by a thorough analysis of all cases observed. Unfortunately, this is not always possible and was not done in my own case as I received the tissue from the physiological department of Loyola University. I attempted to reproduce this condition in male rabbits by blocking the perivesicular lymphatics by injection of liquefied paraffin, followed by a caustic destruction of the bladder mucosa with a concentrated silver nitrate solution and subsequent injection of a bouillon culture of various strains of *B. coli*. The rabbits received moreover intravenous injections of a 10 per cent glucose solution. But the result was negative.

Some Swedish authors could reproduce the intestinal emphysematosis by the injection of gram negative bacteria cultured from the lesions of human material. I believe that the cystitis emphysematosa is also due to a bacterial infection and that cultures made from the lesion will help to establish the etiology.

INTRANUCLEAR INCLUSIONS IN YELLOW FEVER. E. V. Cowdry and S. F. Kitchen (by invitation), St. Louis, Mo.

Abstract. Intranuclear inclusions like those described by Torres in the liver cells of monkeys infected with the Brazilian virus of yellow fever were likewise found in fifteen monkeys inoculated with the African virus of the disease. They were also observed, though less abundantly, in human cases both from the Americas and from Africa. Moreover, the character of the intranuclear inclusions is such as to lead us to classify the virus of yellow fever with other filterable viruses which produce distinctive nuclear inclusions.

Discussion

(Dr. E. B. Krumbhaar, Philadelphia.) Dr. Cowdry's paper is open for discussion.

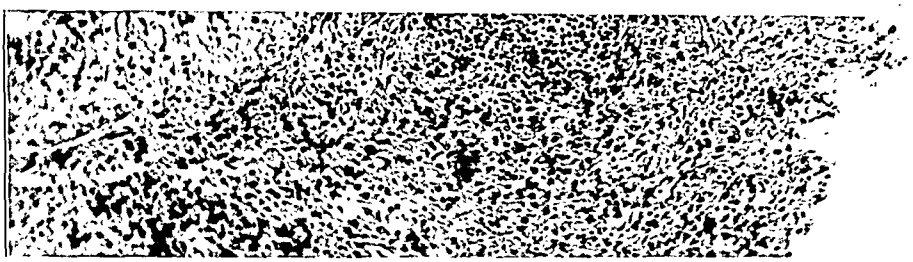
I should like to ask Dr. Cowdry if he would venture any statement as to his ideas on the production of these inclusions in yellow fever and in this group of conditions.

(Dr. Cowdry, closing.) I do not wish to commit myself, but we have no reason to suppose that the inclusions are anything other than a degeneration, what is called an oxychromatic degeneration. We have looked for materials in the

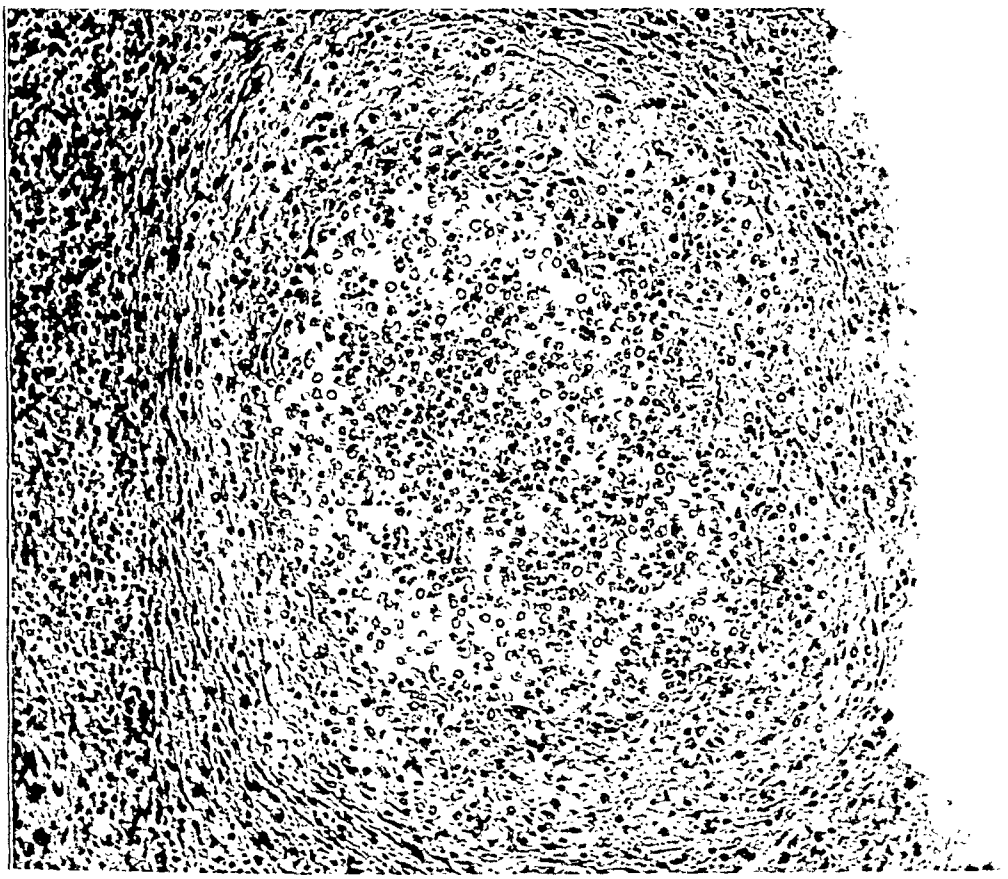
nucleus which should not be there, which might be conceived to come from without, but we have not found them. We have tested the microchemistry of the inclusions as far as we can go, and have found that they do not contain any detectable amount of massed iron as revealed by the MacCallum–Bensley method. Neither do they contain thymonucleic acid. I think I have nothing further to add. It is just a case of a peculiar change in the nucleus which we have associated with cases of yellow fever from these various sources and which we have not been able to observe in lesions in any other disease.



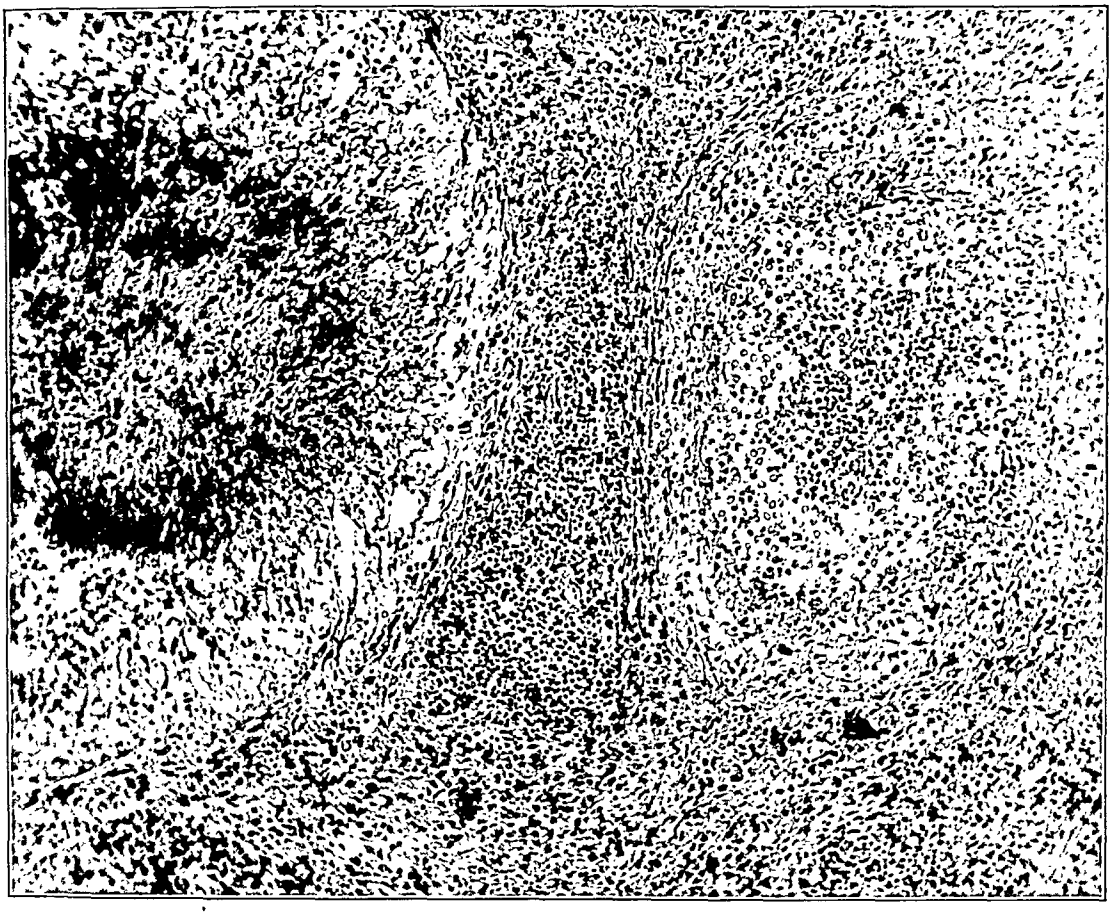
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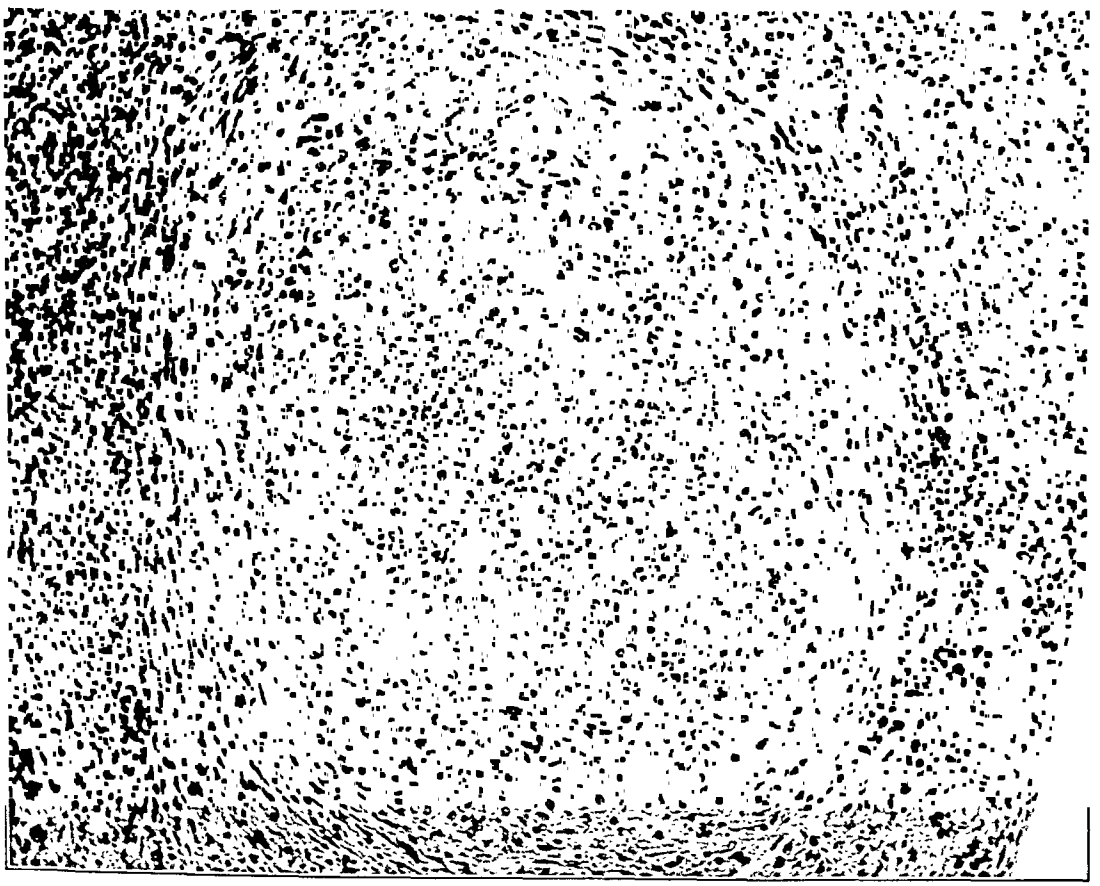
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Multiple Focal Splenitis of Guinea Pigs

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THE FINER HISTOLOGY OF THE NORMAL GLOMERULUS*

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INTRODUCTION

The object of this paper is to review the literature and to present some new facts regarding the histology of the normal human renal glomerulus.

I. REVIEW OF LITERATURE

Bowman's Capsule: In 1688 Malpighi discovered the renal glomerulus by means of injections through the renal artery. He considered it a gland which might possibly give origin to the uriniferous tubule. Schumlansky, 1782, suggested in a diagram some connection between glomeruli and tubules. Littre, 1705, and Johannes Müller, 1830, were the first to describe the capsule. Huschke, 1828, and Müller believed the capsule to be closed except where the vessels entered. Bowman, 1842, proved the union of tubule and glomerulus by a method of successive injection of saturated solutions of potassium bichromate and lead acetate. In both human and animal kidneys the precipitate which formed in the vessels often burst through the glomerular tuft and passed down the tubule. He described the capsule as consisting of an external tunic of transparent homogeneous tissue lined by epithelium. These epithelial cells were non-ciliated, flat and continuous with those of the convoluted tubules. These findings were confirmed in 1845 by Bidder who also noted the continuity of the capsular and tubular basement mem-

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branes. Roth, 1864, and His, 1865, were the first to demonstrate cell boundaries with silver preparations. Ludwig, 1872, described the capsule as consisting of "a mosaic of cells closely resembling those composing the wall of the blood and lymphatic capillaries with a little connective tissue around the outer surface." Drasch, 1877, saw that the nuclei of adjacent cells lay side by side in eccentric positions. Herring, 1900, after a comprehensive review of the embryology of the kidney, concluded that the cavity of Bowman's capsule should be regarded as a body cavity, especially differentiated for urinary secretion.

Mall, 1896, showed the capsular basement membrane to be composed of longitudinally and circularly disposed reticular fibrils, which when very dense and compressed, gave a homogeneous appearance. They anastomosed with the fibrils of adjacent tubules. In spite of these facts, von Ebner in 1902 described the membrane as structureless and glassy. In 1902 Mall was able to show that there were two basal membranes, an inner one homogeneous and structureless, applied to the bases of the epithelial cells, and an outer one interlacing and fibrillar. The latter was part of the reticular stroma of the kidney. Rühle, 1897, Russakoff, 1908, and Corner, 1920, confirmed Mall on these points. Corner believed the fibrillar membrana propria to be produced by the endothelium of adjacent capillaries. von Frisch, 1915, although agreeing that the tubules have a double basement membrane, definitely stated that the capsular membrana propria is a structureless glassy skin. Schäffer, 1927, concluded that the membrane must be considered as a cuticular excretion of the epithelial cells.

Glomerular Endothelium: The history of the cytology of the glomerular tuft begins with Bowman, 1842. He described the vascular loops as lying naked within the lumen of the capsule, held together only by their mutual interlacement. "The Malpighian capillary system stands alone among similar structures in being bare." Among his supporters were Johnson, 1850, Frerichs, 1851, and Henle, 1873. Henle stated: "The vessels in the glomerulus have the calibre of capillaries. Their framework is a structureless wall possessing elliptical nuclei." Many attempts were made to find cell boundaries between these capillary nuclei. Chrzonszczewsky, 1864, Ludwig, 1872, Drasch, 1877, Ribbert, 1879, Hortoles, 1881, and Buday, 1906, after numerous silver injections, were unsuccessful although they

could easily demonstrate boundaries between the endothelial cells of the vas afferens and vas efferens. Langhans, 1879, described the endothelial layer as having very few nuclei. Eliaschoff, 1883, found that these nuclei were more conspicuous in rabbits than in other animals. In 1886, Nussbaum demonstrated cell boundaries in the endothelium of the frog's glomeruli. These cells projected into the lumen and were very distinct in cross-section. von Moellendorff, 1927, described the endothelium of the human glomerulus as a fine layer with a few nuclei and visible cytoplasm only where such nuclei lay. He could not find cell boundaries.

Glomerular Epithelium: The epithelial covering of the glomerular tuft was first described by Gerlach in 1845 and 1848. By injections through the ureter he was able to show that the vascular loops were separated from the capsular space by cells which he believed were the essential elements of a gland. These cells formed a layer over the tips and crevices of the glomerulus. Bidder, 1845, suggested but could not prove conclusively the presence of glomerular epithelium. Carus, 1850, von Wittich, 1851, Koelliker, 1854, McDonnell, 1855, Chrzonszczewsky, 1864, Duncan, 1867, and Toldt, 1874, all supported Gerlach. In 1857 Isaacs injected glomeruli by way of the ureter and tubules until the capsules ruptured. With a dissecting microscope he examined through the hole in the capsule the epithelial covering of the loops. His illustrations showed how much larger these cells were than those lining Bowman's capsule. Moleschott, 1862, measured the glomerular and capsular epithelial cells and found the diameters to average 0.01 and 0.006 mm., respectively. Steudener, 1864, saw a fine covering over the glomerular loops but could not recognize it as epithelium. Schweigger-Seidel, 1865, and Seng, 1871, observed the presence of epithelium over the tips of the loops in the embryo. Ludwig, 1872, saw that the walls of the capillaries were separated from the capsular contents by "a layer of not very well defined cells with spherical nuclei." He was not sure that these were continuous from lobule to lobule. R. Heidenhain, 1874, injected the renal arteries of cats, birds, rats, field mice, rabbits, guinea pigs, sheep, pigs and cows, and found the epithelial cells forming a continuous layer over and between the loops. His work was confirmed by the findings of Frey, 1875, Langhans, 1879, Ribbert, 1879, and Nauwerck, 1884. Ribbert stated that the numerous nuclei observed in a normal glomerulus were for the most

part epithelial. Drasch, 1877, and von Ebner, 1902, believed this epithelium was in the form of a syncytium. Ziegler, 1879, preferred to consider it as endothelium comparable to the lining of the serous cavities. Nussbaum, 1886, clearly distinguished the large glomerular epithelium from the smaller endothelium. He believed the epithelial cells were often multinucleated and that amitosis occurred in them. He recognized the continuity of glomerular and capsular epithelium. Hansemann, 1887, with Isaac's technique isolated the glomerular epithelium and described the cells as flat, often multinucleated, and taller than those lining the capsule. He pointed out the more rapid postmortem digestion of the epithelial than of the endothelial cells. In 1900, Herring's review of the development of the glomerulus explained completely the position and derivation of glomerular epithelium. In a 1909 Harvey lecture, Huber stated that the columnar glomerular epithelium of the embryo became pavement epithelium in the adult. Mertz, 1918, in a quantitative study of the cells in the average 10 micron section of a human glomerulus, was unable to find sufficient morphological difference between endothelium and epithelium to count them separately. Löhlein, 1910, Gross, 1919, Fahr, 1925, Munk, 1925, and Elwyn, 1926, all described glomerular epithelium and its pathological changes. Gross preferred to think of it as a syncytium rather than true epithelium. Fahr stated that glomerular epithelium was so closely related to tubular that it would seem to be secretory. Zimmerman, 1911, 1915 and 1923, demonstrated boundaries between these cells in cats and hedgehogs, but preferred to call them pericytes. von Moellendorff, 1927, described the "deck" cells or glomerular epithelium as morphologically similar to the adventitial cells or pericytes of the capillary bed. He believed these cells have long, branching, anastomosing processes which form a network over the glomerular loops. He ascribed to their mesenchymal origin, their ability to take the form of adventitial cells and to transform into free round cells in pathological conditions. Vimtrup, 1928, adhered to the older conception of an epithelial covering closely applied to the surface of each capillary loop. Volterra, 1928, described these cells as perithelial but did not see any processes on them. He concluded that the cell branches described by von Moellendorff belonged to the glomerular basement membrane and were a part of the connective tissue adventitia of the capillary tuft. Bargmann, 1929, studied the glomeruli of many species and believed

with von Moellendorff that the branching processes belonged to the "deck" cells.

Glomerular Basement Membrane: The possibility of a membrana propria in the glomerular loops was evidently considered by Bowman and rejected for he wrote: "The capsular basement membrane is perforated by the afferent and efferent vessels and is certainly not reflected over them." Seng, 1871, saw and illustrated the presence and continuity of the glomerular and capsular basal membranes. Toldt, 1874, confirmed this finding in both embryonic and adult human glomeruli. Drasch, 1877, after injection of silver nitrate into the glomerular vessels, saw a formation which depended on a porous condition in a membrane. Langhans' illustrations, 1879, show a basement membrane which he described as a very fine doubly-contoured border. Nussbaum, 1886, after studying the embryology of the malpighian body, searched unsuccessfully for a glomerular membrane and concluded that the wall of a glomerulus had only two layers, endothelial and epithelial. Mall, 1896, after boiling kidney tissue in water, digested frozen sections with pancreatin and found a homogeneous striped membrane still present in the glomerular loops. Rühle, 1897, injected a rabbit's kidney with alcohol and was able to see an indistinct membrane in the glomerular loops. Fibers from the capsular basement membrane passed into the glomerular membrane. During digestion with pancreatin the epithelial and endothelial cells disappeared leaving a regularly striped fibrous membrane showing numerous fine pores. This confirmed the work of Drasch. Regaud and Policard, 1903, studying the kidneys of ophidians stained with alum hematoxylin, demonstrated a thin homogeneous membrane separating the endothelium from the synctyial epiglomerular layer. Hueter, 1908, stained the glomerular basement membrane with Ribbert's connective tissue stain. Gross, 1919, found that the membrane took a gray or brown color with Heidenhain's iron hematoxylin. Ohmori, 1921, obtained a very clear differentiation of the membrane with a rein blau-picric acid dye. Hung, 1922, was able to stain it distinctly if the tissue were boiled before fixation and later put through hematoxylin-eosin, Weigert's elastic tissue stain or Mallory's anilin blue. Krauspe, 1922, using Bielschowsky's method found the glomerular wall to consist of homogeneous gray or black membrane lined inside by endothelium and outside by epithelium. He saw the transition into Bowman's base-

ment membrane but could not detect the fine streaking mentioned by Rühle. Schäffer, 1927, noted the presence of the glomerular membrane but did not consider its structure to be fibrillar. von Moellendorff, 1927, demonstrated a homogeneous membrane with Heidenhain's azan-carmine and with his own eosin-methylene blue. Volterra, 1928, described the membrane as a continuous, argyrophil, net-like, reticular adventitia resting on endothelium but not formed by it and adherent to the pericytes. The processes which von Moellendorff assigned to the "deck" cells were, according to Volterra, the connective tissue fibrils of the membrane. Bargmann, 1929, described the glomerular membrane as a deep black or brown granular line when impregnated with silver.

Glomerular Connective Tissue: Key, in 1865, described star-shaped cells which he considered connective tissue between the glomerular loops. Ludwig, 1872, stated that the capillaries of the tuft had an investment of connective tissue. Klebs, 1863, and 1889, thought there were small numbers of such cells in all glomeruli. Toldt, 1874, saw central cells in the crevices of the tuft which were different from the epithelial cells. However, as the glomerulus developed and became spherical these connective tissue elements were no longer visible. In 1880, Waller confirmed Key's finding. In normal cat glomeruli he distinguished epithelium from "connective tissue corpuscles" which were situated between the lobes and stained with logwood after carmine and gelatin injections. He studied the glomeruli as a whole under a dissecting microscope and considered these cells to have a special affinity for vegetable coloring matter. Greenfield, 1880, also believed there were connective tissue cells within the glomerular tuft and Ribbert, 1879, described their nuclei as being small, spindle-shaped, and more intensely stained with carmine than the larger round finely granular epithelial nuclei. Opposing this view were Drasch, 1877, Langhans, 1885, Nauwerck 1886, Nussbaum, 1886, Hansemann, 1887, Orth, 1893, Mertz, 1918, and von Moellendorff, 1927. Nussbaum concluded that the connective tissue which he expected to find, in consideration of the embryological development, must have been resorbed. Herring, 1900, Mallory, 1914, and Vimtrup, 1928, have stated that some connective tissue persists around the hilum probably continuing into the glomerulus to bind the capillaries together.

With regard to the presence of reticulum within the tuft, John-

ston, 1899, believed these fibrils in Bowman's capsule were not reflected over the glomerulus. As the afferent vessel pierced the capsule, fibrils arose which penetrated the glomerulus, passing in all directions between its capillaries. These became less and less numerous as the periphery was approached. Luna, 1920, using the method of Achūcarro–del Río-Hortega, saw an occasional adventitial sheath in the glomerular capillaries. Corner, 1920, decided that the endothelium of the glomerular tuft was never provided with a reticulum. Krauspe, 1922, employing the Bielschowsky–Maresch technique, after a study of 150 human kidneys, could not find *gitterfasern* in the glomerular capillaries. Foot, 1925, decided that no reticulum was seen in the human renal tuft under ordinary circumstances. Allen, 1927, using silver impregnation concluded that occasional glomeruli showed reticulum fibrils in the tuft, usually near the pedicle. A few fibers radiated peripherally with no constant relation to reticulum cells or capillary endothelium. Bargmann, 1929, saw an argyrophil network deep in the capillary wall but he could not say to what it belonged.

Shape and Size of Glomerulus: Vimtrup, 1928, described the human malpighian body as oval with an average diameter of 200 microns as compared with Fahr's figure of 237 microns.

Mertz, 1918, found that the total number of cells in 10 to 15 micron sections through the greatest diameter of normal glomeruli was constant, and at most 145. Of these 3 to 30 were leucocytes, leaving 130 endothelial and epithelial. von Moellendorff, 1927, did not make a quantitative study but concluded that there were at least ten times as many epithelial as endothelial cells. Bargmann, 1929, made the same observation.

Development of the Glomerulus: According to Herring, 1900, and Huber, 1909, the malpighian body, convoluted tubules, Henle's loops, junctional tubules, connective tissue framework and capsule arise from the kidney blastema, a mass of cells formed from the intermediate cell mass. Blood vessels are partly of the same origin and partly branches from the renal artery. Malpighian bodies and their tubules begin to appear at the end of the second month. Each arises as a solid mass of cells which acquires a lumen and becomes S-shaped. The lower limb of the "S" becomes the malpighian body, the upper and middle limbs form the convoluted tubules. The convex side of the lower limb is formed by the epithelium which later lines

Bowman's capsule, the concave side by the epithelium which later covers the glomerulus. The glomerulus is at first non-vascular and is formed by a thickening of the epithelium rather than by an invagination. The capillaries form *in situ* from mesenchymal connective tissue cells close to the base of the glomerulus and are joined by a branch of the renal artery. The division of the glomerulus into lobes is due to the penetration of the epithelial cells between the capillary loops.

Summary

To summarize the present status of the histology of the normal glomerulus, it may be said:

1. The shape is oval and the diameter varies between 200 and 237 microns.

2. The capsule consists of a basement membrane lined by flat epithelial cells which become taller near the opening into the tubule. The structure of the membrane and its relation to the glomerular membrane are not agreed upon.

3. The wall of the glomerular loop contains a basement membrane whose porosity and fibrillar or homogeneous character are unsettled subjects.

4. Within the glomerular membrane are a few endothelial cells between which, boundaries have not been definitely established.

5. Outside the glomerular membrane is a layer of cells whose epithelial, adventitial and syncytial characters are controversial points.

6. The presence of reticular fibrils in the tuft beyond the wall of the vas afferens is indefinite as is also the existence there of connective tissue cells.

II. Methods Used and Results Obtained in this Study

Fixation of Tissues: In general the results were best following Zenker or Helly fixation. However, most of the tissues available for this work had already been fixed in 10 per cent formalin. Davidoff, 1928, described a method of taking nerve tissue from formalin to Zenker. This was tried on kidney tissue and was found to work very successfully. The technique may be applied to the tissue in blocks or to paraffin sections.

(*a*) For paraffin sections of formalin-fixed tissues.

1. One hour in ammonia (40 drops to 10 cc. of water).
2. One hour in running water.
3. One hour in Zenker's or Helly's fluid.
4. One hour in running water.

(*b*) For blocks of formalin-fixed tissue.

1. Two days in paraffin oven at 40° C in ammonia (40 drops to 100 cc. of water).
2. Twenty-four hours in running water.
3. Zenker twelve hours, or Helly five hours.
4. Twelve hours in running water.

Staining of Tissues: The following stains have been used: Hematoxylin and eosin, Mallory's eosin-methylene blue, methyl green-pyronin, Van Gieson, Weigert's elastic tissue stain, Ohmori's rein blau-picric acid, Mallory's anilin blue, Heidenhain's azan-carmine modification of Mallory's anilin blue, Lee-Brown modification of Mallory's anilin blue, Bielschowsky–Maresch, Bielschowsky–Perdrau (Bailey and Hiller), Bielschowsky–Ferguson, Bielschowsky–Foot, Hortega–Foot silver carbonate.

The technique for the three stains which were found to be the most useful is as follows:

(*a*) Mallory's anilin blue (Heidenhain's azan-carmine modification*).

1. 1 per cent azan-carmine G (1 gm. in 100 cc. of water, heat, cool, filter at room temperature and add 1 cc. glacial acetic acid). Thirty to forty minutes in this stain in the paraffin oven at 56° C.
2. Wash in water.
3. Differentiate in anilin alcohol (1 cc. anilin oil in 100 cc. 95 per cent alcohol). Watch under the microscope until the nuclei are red and the cytoplasm pale pink. This step requires from one to three minutes, depending on the thickness of the sections.
4. Remove anilin with acid alcohol, about one minute.
5. Three hours in 5 per cent phosphotungstic acid.
6. Wash quickly in water.
7. Three to six hours in:

anilin blue	0.5 gm.
orange G	2. gm.
glacial acetic acid	8. cc.
distilled water	100. cc.

Boil, cool and filter. Dilute one-half with water.

8. Wash in water.
9. Differentiate in *absolute alcohol, watching under the microscope.*
10. Xylol.
11. Balsam.

* Some changes were made in this technique so the directions do not correspond with the original as given by Heidenhain.

Nuclei orange-red, cytoplasm pink, connective tissue and reticulum blue, fibrin red.

(*b*) Ohmori's rein blau-picric acid.

1. Stain the nuclei with acid fuchsin or lithium carmine at least one-half hour.
2. One minute in rein blau-picric acid (to a concentrated aqueous solution of picric acid add a concentrated aqueous solution of rein blau until a dark green color appears).
3. Differentiate in absolute alcohol.
4. Xylol.
5. Balsam.

(*c*) Mallory's anilin blue, Lee-Brown modification.

1. Phosphomolybdic acid 1 per cent for thirty seconds.
2. Distilled water for 1 to 2 minutes.
3. Mallory's stain:

anilin blue	0.5 gm.	for 1 to 5 minutes
orange G	2. gm.	
phosphomolybdic acid	2. gm.	
distilled water	100. cc.	

4. Distilled water for 2 to 5 minutes.
5. Phosphomolybdic acid 1 per cent for 30 seconds.
6. Distilled water for 1 to 2 minutes.
7. Dehydrate, clear and mount.

The Capsular Basement Membrane: The outer layer of Bowman's capsule is composed of argyrophil fibrils which anastomose with the intertubular stroma of the kidney and with the reticular covering of the tubules. The inner layer of Bowman's capsule is an apparently homogeneous membrane which stains red with Van Gieson, blue with Ohmori and dark blue with the Mallory–Heidenhain azan-carmine. This structure is continuous with the glomerular and tubular basal membranes.

The Capsular Epithelium: These cells form a single layer completely lining the capsule. Their cell borders are distinct and as would be expected from their embryological origin they are directly continuous with the glomerular and tubular epithelium. Their nuclei are elongated, with finely dispersed chromatin and one or more large nucleoli.

Glomerular Epithelium: These are the cells which, in the embryo, form the concave side of the lower limb of the "S" (the convex side of the capsular space). During fetal life and early childhood the arrangement is columnar, this gradually becoming flattened in the adult.

These cells form a complete single layer of covering over the tips and crevices of the capillary tufts. At the hilum they are continuous with the capsular, and thus with the tubular epithelium. They lie outside the glomerular basement membrane which separates them from the endothelium. The arrangement is not syncytial and cell boundaries are easily seen. They are much more numerous than endothelium.

Each cell has abundant cytoplasm and a large oval nucleus situated far from the base. The chromatin is finely dispersed near the nuclear membrane and there are one or two nucleoli. The cytoplasm takes a basophilic tint with Mallory's eosin-methylene blue. The morphology is very similar to that of the capsular epithelium.

Glomerular Basement Membrane: The S-shaped mass of cells destined to form the glomerulus and its tubule is surrounded by a membrane. The portion of this structure covering the concavity of the lower limb of the "S" becomes the glomerular basement membrane. It is closely associated with the glomerular epithelium and is present before the glomerular capillaries appear.

In sections stained with anilin blue or with rein blau the membrane is a single deep blue line lying between endothelium and glomerular epithelium and forms the most striking portion of the glomerular capillary wall. It does not stain with any of the silver impregnation methods used.

For the most part its structure seems to be homogeneous. However, near the hilum there is sometimes a lamellated appearance. Whether the membrane is fibrillar or whether the extra fibrils belong to the connective tissue cells of the vascular pedicle has not yet been determined.

The vas afferens with its supporting framework passes well into the center of the glomerulus before it breaks up into capillaries. Its subendothelial connective tissue seems to fuse with the glomerular basement membrane.

The lumen of each glomerular branch of the afferent vessel is narrowed at fairly regular intervals with openings into numerous small capillary loops. At each of these orifices the basement membrane appears thickened and contracted while in all other portions of the capillary wall it is thin and quite uniform.

The continuity of the glomerular capsular and tubular membranes is evident in any fetal or adult kidney stained with anilin

blue or with rein blau. While differentiating these sections in absolute alcohol the glomerular basement membrane decolorizes before any other membrane or connective tissue in the kidney.

Glomerular Endothelium: These cells are continuous with the endothelium of the vas afferens and efferens and are situated inside the glomerular basement membrane. Their nuclei are widely separated and are much less numerous than those of the epithelium. A continuous layer of cytoplasm lining the membrane and connecting adjacent cells is rarely visible. The nuclei are small and elongated with a thick nuclear membrane and coarse, evenly distributed chromatin. The small amount of cytoplasm at each end of the nucleus makes the cell appear stretched and flattened against the basement membrane. The question of cell boundaries has not been investigated.

There is another type of cell in the wall of the capillary which is probably endothelial also. It is always situated inside the passageway between loops or adjacent to the orifice. In this way it lies next the thickened portions of the glomerular membrane and seems to be almost surrounded by it. This cell cannot be considered epithelial because it is inside the basement membrane. With the stains used in this study the nucleus cannot be distinguished from that of typical endothelium. Its cytoplasm is extremely minute, perhaps because of its position in the constricted portion of the capillary. There is a possibility that it is a connective tissue cell enclosed in the membrane. Further studies on this point are in progress.

Connective Tissue Cells: These accompany the afferent vessel almost to the center of the glomerulus and are always visible in sections through the greatest glomerular diameter. Reticulum argyrophil fibrils also enter at the hilum, pass into the glomerulus and end in finer fibrils or very small clusters. They do not accompany the basement membrane of the capillary loops but seem rather to be associated with the afferent vessel.

Summary

The following conclusions have been reached after studying oil immersion fields of tissues stained as described previously.

1. The glomerular, capsular and tubular basement membranes are continuous and stain red with Van Gieson, blue with Ohmori and

dark blue with Mallory–Heidenhain azan-carmine. The capsule and tubules have an additional external, reticular, argyrophil covering. The glomerular basement membrane is thinner than that of the capsule and tubules. Pores have not been demonstrated, nor any elastic fiber content.

2. The glomerular, capsular and tubular epithelium are continuous. The glomerular epithelial cells have abundant cytoplasm and large vesicular nuclei with one or more prominent nucleoli. These are the only cells which lie outside the glomerular basement membrane. They form complete covering for the loops following closely both tips and crevices. They are arranged in a single layer and not in a syncytium.

3. The glomerular endothelium lies inside the basement membrane and therefore cannot be confused with epithelium. The cytoplasm is very small in amount and the nuclei have a thick nuclear membrane enclosing much coarse chromatin. Endothelial cells are much less numerous than epithelial.

There are cells, probably endothelial, which lie inside the glomerular basement membrane but are partially surrounded by it. Their nuclei seem to be identical with those of typical endothelium and they have very little cytoplasm. Their situation at the orifices between loops makes examination very difficult. Further study to decide their nature is in progress.

4. Connective tissue cells and reticulum fibrils pass in at the hilum accompanying the vas afferens until it subdivides near the center of the glomerulus.

BIBLIOGRAPHY

See following paper for bibliography.

DESCRIPTION OF PLATE

PLATE 104

FIG. 1. Diagram of section through greatest diameter of a normal glomerulus. C. tub. = convoluted tubule; aff. a. = afferent artery; c.t. = connective tissue; c.b.m. = capsular basement membrane; c.ep. = capsular epithelium; gl.ep. = glomerular epithelium; gl.b.m. = glomerular basement membrane; end. = endothelium; end. l. = type of endothelial cell found at passageway between loops; r.b.c. = red blood cell; l. = leucocyte; t. = thickening of glomerular basement membrane often seen at orifice into another loop.

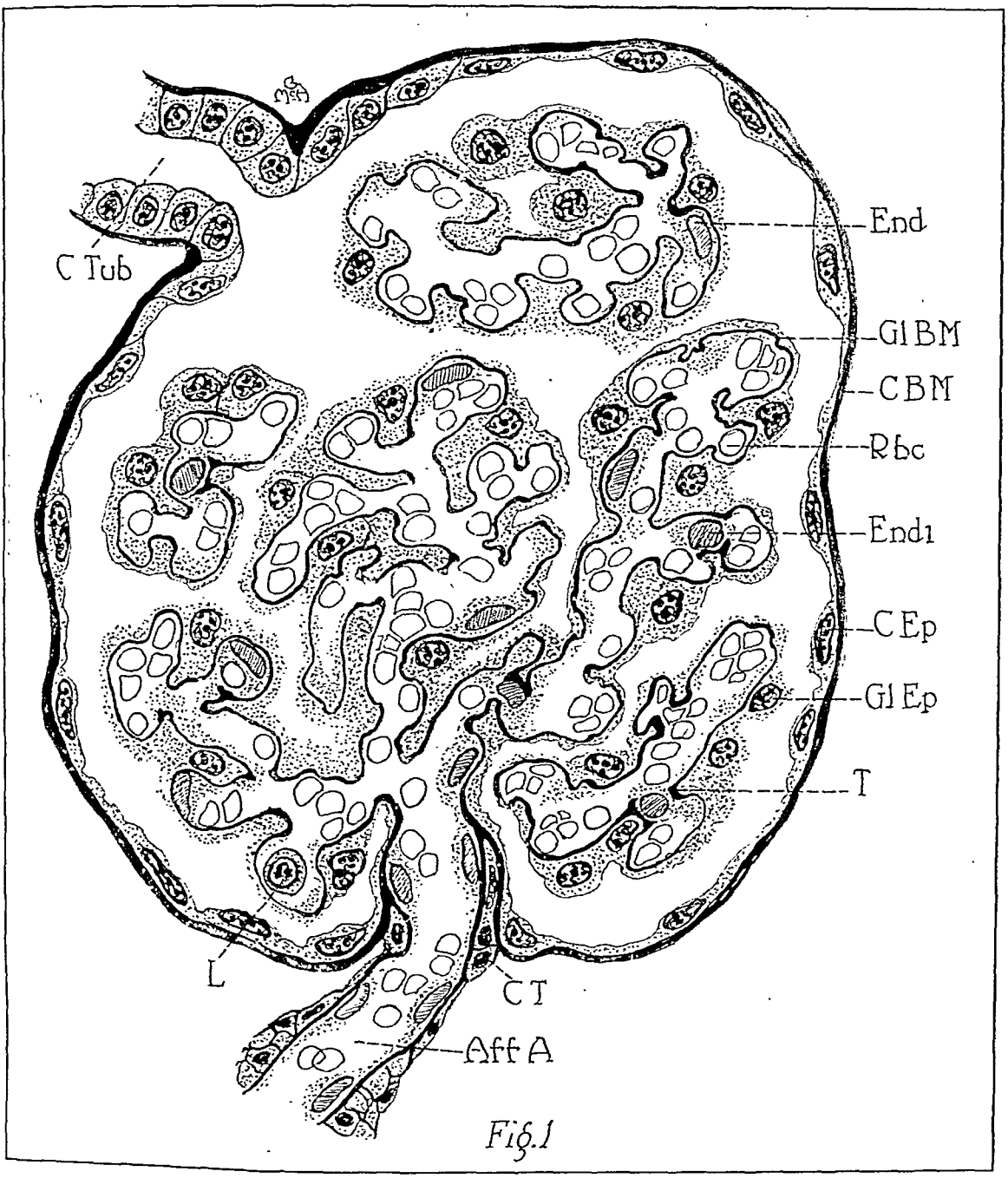


Fig. 1

THE CYTOLOGICAL CHANGES OCCURRING IN THE GLOMERULUS OF CLINICAL GLOMERULONEPHRITIS *

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INTRODUCTION

The object of this paper is to review the literature and to present some new facts regarding the cytological changes occurring in the glomerulus of clinical glomerulonephritis, acute and chronic.

I. REVIEW OF LITERATURE

Richard Bright in 1827 considered the renal lesions in nephritis to be of an inflammatory character. The work of Christison, 1839, Rayer, 1840, Johnson, 1847 and 1850, Frerichs, 1851, Virchow, 1852, and Wilks, 1853, directed attention toward the tubular changes of fatty degeneration, desquamation and proliferation. However in 1850, Johnson observed changes in the glomerular capillary walls in chronic renal disease. From that time on the glomeruli as well as the tubules were studied.

Glomerular Endothelial Reactions: Since most of the very early investigators were convinced of the interstitial and tubular nature of nephritis, very few studies were made of glomerular endothelium. Johnson, 1873, noted that the nuclei in the walls of the glomerular capillaries were abnormally conspicuous. Langhans, 1879, was the first to call attention to endothelial proliferation. Hortoles, 1881, describing a case of scarlet fever, believed the multiplication of the endothelial cells was due to their reversion to an embryonic state. Friedländer, 1883, saw proliferation of "the endothelial tube." Aufrecht, 1886, found a swelling but no increase in number of these cells. In 1885 Langhans' detailed description of the glomerular capillaries in acute nephritis supported what he believed to be the essential lesion, an almost complete occlusion of the loops by endothelial proliferation. Welch, 1886, corroborated this statement. Nauwerck,

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1886, saw endothelial cells with several nuclei and supposed their multiplication was so extensive that they were cast off into the lumen. Ribbert, 1879, and Hansemann, 1887, because of the scarcity of endothelial nuclei in normal glomeruli could not believe they were the source of proliferation in nephritis. Klebs, 1889, differing from his earlier publication, stressed the swelling of endothelium as part of the lesion. Sorenson, 1891, reached the same conclusion. Councilman, 1897, reviewing forty-nine cases of acute and subacute nephritis, confirmed Langhans in the conclusion that most of the nuclear increase was endothelial. Herring, 1900, made a similar observation. Löhlein, 1910, described a proliferation and "net-like arrangement of cellular elements of the endothelial type." Kaufmann, 1911, saw a marked increase in endothelium with desquamation into the capillary lumen. Gaskell, 1911, observed a homogeneous swelling of the capillary wall cells with subsequent fatty degeneration. Aschoff, 1913, Fahr, 1914, Mallory, 1914, MacCallum, 1916, Dietrich, 1917, and Jungman, 1917, all explained the narrowed lumina of the capillary loops as due to endothelial proliferation. Mertz, 1918, in his quantitative study, concluded that between the ninth and twenty-first days of nephritis the endothelial cells at least doubled in number, their increase being proportional to the severity of the disease. Since he believed the number of glomerular epithelial cells to be constant, his figures for endothelial increase are inexact. Herxheimer, 1916, and 1918, in a comprehensive review of war nephritis, stated that the first morphological change was growth of endothelium. Gross, 1919, confirmed Kaufmann as to the proliferation and desquamation of these cells. Mallory, 1914, Ohmori, 1921, Bell and Hartzell, 1922, Munk, 1925, Kuczynski and Dosquet, 1926, Elwyn, 1926, and Hückel, 1928, all described varying degrees of increase in endothelium. MacCallum, 1921, said "the glomerular tufts become greatly enlarged through the appearance of quantities of cells in the lumina of the capillaries, which are not polymorphonuclear leucocytes, but look more like endothelial cells. At least they are mononuclear cells with indistinct outline, so fused together that their origin can hardly be made out." Fahr, 1925, observed such marked proliferation and desquamation that a two to three-celled layer was formed in the capillaries.

Mallory, 1914, Herxheimer, 1916, Gross, 1919, and Elwyn, 1926, have reported mitoses in glomerular endothelial cells.

Intracapillary Leucocytes: The opinion has been uniformly held that a part of the glomerular cell increase was polymorphonuclear. However, Buhl, 1878, and Waller, 1880, decided the leucocyte accumulation was the outstanding feature. Langhans, 1885, stated that leucocytes helped to occlude the glomerular capillaries, their cytoplasm fusing with that of the proliferating endothelium. Cornil, 1879, and Hansemann, 1887, believed the so-called endothelial multiplication was merely an aggregation of leucocytes. Obrzut, 1888, and Ribbert, 1888, both agreed with Buhl and Waller that the leucocytes could later form connective tissue. Gräff, 1916, Herxheimer, 1916 and 1918, and Mertz, 1918, studied in detail the polymorphonuclear exudate. Mertz concluded that in very early stages these cells were increased up to five times their normal number. Aschoff had pointed out that leucocytes in glomeruli might not be due to inflammation, but merely a condition seen in the agonal stage in circulatory disease. After a study of control cases Herxheimer disagreed. Mallory, 1914, called attention to the participation of endothelial leucocytes (large mononuclears or monocytes) in the exudate in slowly progressive cases. He suggested that some wandered in from the blood stream, while others were desquamated endothelium. Bell and Hartzell, 1922, considered the leucocytic reaction polymorphonuclear in type.

Glomerular Intracapillary Network: Cellular and non-cellular types of network within the glomerular loops have been described. In 1849, Rokitansky explained the interference with the glomerular circulation on the basis of a fibrino-albuminous deposit within the capillaries. According to Langhans, 1885, the growing endothelium formed a reticulum within the loops and led to closure of the lumina. To Hansemann, 1887, this conception was erroneous and was apparent only in very thick sections. Klebs, 1889, described obstruction of the capillaries by a syncytium composed of endothelium, leucocytes and masses which stained red with eosin. Thrombotic occlusion of the loops by endothelial cells and leucocytes or by granular masses was seen by Friedländer, 1883, and Nauwerck, 1886. Böhm, 1897, and Kaufmann, 1911, also described an accumulation of fine granular detritus coming from endothelium and blood corpuscles. Fibrin excretion was prominent in the scarlet fever cases studied by Reichel, 1905. Herxheimer, 1916, believed leucocytes were mechanically held in the loops due to injury of the endothelium. He decided coagulated blood serum was the source of the cytoplas-

mic network described by Aufrecht, 1894, as coming from red blood cells. Mallory, 1914, described fibrin in the loops in the form of threads, a network or solid masses. Löhlein, 1910, and Rochs, 1918, observed a coagulated cytoplasmic meshwork within the vessel lumina. Gross, 1919, found free endothelial cells within the network which stained with cresyl violet and Heidenhain's pale blue but not with Van Gieson or Weigert. Ohmori, 1921, saw a deposition of fibrin flakes on the inside of the glomerular basement membrane. These increased in amount, became confluent and crossed the loops. MacCallum, 1916, ascribed the capillary occlusion to the proliferating endothelium and to indefinite foamy masses of cytoplasm. Aschoff, 1921, mentioned the presence of fibrin and nuclei in the cytoplasmic glomerular network. Excessive endothelial cytoplasm alone obstructed the lumen in a case described by Fahr, 1925.

Glomerular Epithelium: Klein, 1877, was the first to mention an increase in nuclei of cells "probably epithelial" covering the tuft. Langhans, 1879, saw such swelling of the cytoplasm and nuclei of these cells that they were attached only by narrow pedicles to the loop wall. When the increase in size progressed far enough they were desquamated in large numbers. Weigert, 1879, and Ziegler, 1879, observed swelling, degeneration and destruction of glomerular epithelium as one of the earliest changes in acute nephritis. Greenfield, 1880, and Saundry, 1880, found proliferation of epithelium, which they called endothelium because they considered it comparable to the peritoneal and pleural lining cells. Litten, 1878, and Dunin, 1883, believed the degenerating epithelium liberated chemicals injurious to the vessel walls and causing inflammation, although Dunin also stated that vascular changes could occur independently of epithelial disease. Aufrecht, 1886 and 1894, described swelling but not proliferation of glomerular epithelium. Kiener and Kelsch, 1882, Cornil and Brault, 1883, Nauwerck, 1886, and Klebs, 1889, all saw multiplication, degeneration, necrosis and desquamation of these cells until the capillary loops were bare. Welch, 1886, and Ribbert, 1888, noted these same epithelial changes. Hansemann, 1887, explained the albuminuria as due to the nakedness of the capillary wall following the desquamation. Ernst, 1893, thought fibrin was able to pass through the glomerular loop after the death of the epithelium. Orth, 1893, and Böhm, 1897, described degeneration of these cells. Herring, 1900, concluded glomerular epithelium was

highly organized and therefore easily injured. Herxheimer, 1909, agreed with Aufrecht, 1886, and with Engel, 1901, that the primary injury in glomerulonephritis is epithelial. Löhlein, 1910, found slight swelling of the epithelium in early stages which passed on to desquamation in severe cases. Kaufmann, 1911, saw both proliferative and degenerative changes. Herxheimer, 1916, observed swelling and the formation of giant cells in this epithelium. Mertz, 1918, on the other hand, decided that the number of epithelial cells was a constant which did not increase. Gross, 1919, felt that endothelial proliferation preceded epithelial degeneration. Mitoses were mentioned in the glomerular epithelium by Mallory, 1914, MacCallum, 1916, Fahr, 1925, Munk, 1925, and Kuczynski and Dosquet, 1926; all showed illustrations of the changes occurring in these cells.

Hyaline granular degeneration of glomerular epithelium similar to that commonly seen in convoluted tubules has been described by Posner, 1880, Aufrecht, 1886, Kaufmann, 1911, Mallory, 1914, Herxheimer, 1916, Gross, 1919, Aschoff, 1921, Fahr, 1925, and Kuczynski and Dosquet, 1926.

Glomerular Connective Tissue Proliferation: Klebs, 1863, stated that in glomerulonephritis the inflammation was situated between the coils of the glomerulus in the small amount of connective tissue there. By 1889, his opinion was modified to the extent that he no longer considered connective tissue proliferation a conspicuous feature. Waller, 1880, confirmed Klebs' original statement because the first nuclear increase occurred between the loops and later progressed toward the tips. Greenfield, 1880, and Böhm, 1897, described a proliferation of connective tissue cells around the vas and between the glomerular loops. Councilman, 1897, and Herring, 1900, thought a few of the new cells were of connective tissue origin. Mallory, 1914, stated that the fibroblasts forming the supporting framework of the capillaries were stimulated to proliferation.

Capsular Epithelial Proliferation: Swelling and proliferation of these cells were first described by Langhans, 1879, Ziegler, 1879, and Greenfield, 1880. The latter two believed the cells to be endothelial with a reaction similar to that of the serous membranes. Saundry, 1880, von Leyden, 1881, Aufrecht, 1886 and 1894, Nauwerck, 1886, Welch, 1886, Ribbert, 1888, Klebs, 1889, Orth, 1893, von Kahlden, 1894, Herring, 1900, Herxheimer, 1909, Löhlein, 1910, Gaskell, 1911, Mallory, 1914, MacCallum, 1916, Aschoff, 1921, Bell and Hartzell,

1922, Fahr, 1925, and Munk, 1925, all confirmed the work of their predecessors on this point.

Hyaline granular degeneration of capsular epithelium has been reported by Aufrecht, 1894, Mallory, 1914, and Fahr, 1925. Mitoses were seen in these cells by Mallory, 1914.

Randerath, 1929, demonstrated anatomically the presence of a pericapsular capillary net. He suggested that bacteria in these capillaries causing an increased permeability of the capsular basement membrane could stimulate the capsular epithelium to proliferation.

Crescent Formation: Langhans, 1879, Nauwerck, 1886, and Fahr, 1925, decided the chief source of the cells in the crescent was desquamated glomerular epithelium. Ziegler, 1879, Greenfield, 1880, Leyden, 1881, Dunin, 1883, and MacCallum, 1916, have ascribed the origin of crescents to capsular epithelium. Saundry, 1880, Welch, 1886, Hansemann, 1887, Orth, 1893, Engel, 1901, Löhlein, 1910, Kaufmann, 1911, Mallory, 1914, Herxheimer, 1916, Aschoff, 1921, and Munk, 1925, have all stated that both capsular and desquamated glomerular epithelium take part in crescent formation. Waller, 1880, and Obrzut, 1888, suggested that emigrated leucocytes changing into connective tissue produced the crescent.

The presence of fibrin in the capsular space and crescent has been reported many times. It was described as a conspicuous feature by Ernst, 1893, Engel, 1901, Löhlein, 1910, Kaufmann, 1911, Mallory, 1914, MacCallum, 1916, Ohmori, 1921, Aschoff, 1921, and Fahr, 1925. Ernst saw a fibrin net between the cells of the crescent and extending into the convoluted tubule. The descriptions of Mallory, Aschoff and MacCallum were very similar. According to Engel, the source of the fibrin was either the blood plasma or the degenerated epithelium. Löhlein explained the fibrin as forming from blood extravasated into the capsular space. Fahr disagreed with this view and described it as an inflammatory excretion. Herxheimer, 1916 and 1918, found no intracapsular fibrin in the war nephritis cases.

Herxheimer described hyaline bodies in the crescent which were not fibrin, which stained yellow with Van Gieson, and which were sometimes inside the cytoplasm of growing epithelial cells. MacCallum also saw hyaline masses in contact with the epithelium and thought they arose from fusion of disintegrating red blood corpuscles which had passed through the capillary wall.

Almost all workers have described coagulated albumin, red blood

cells, polymorphonuclears, desquamated disintegrating glomerular epithelium and fat in the capsular space or in the crescent. Langhans, Gräff and Herxheimer have seen leucocytes passing through the wall of the glomerular loops.

In an explanation of the connective tissue organization of the crescent, Mallory, 1914, stated that fibrin in the capsular space stimulated fibroblasts in the tuft and capsule wherever it touched them. They proliferated and organized until the fibrin was replaced by collagen. MacCallum, 1916, believed the proliferating epithelial cells brought with them a supporting framework of connective tissue.

Changes in Permeability of Glomerular Capillary Wall: The large amount of exudate in the tubules and capsular spaces in nephritis has suggested an increased permeability of the glomerular capillary walls. No one has been able to demonstrate stomata or other morphological evidence of this process. Toynbee, 1846, and Thoma, 1877, showed experimentally that in normal kidneys, injections of a calcium solution and Berlin blue at a pressure of 50 mm. Hg. very rarely went through the loops into the capsular space. On the other hand, in nephritic kidneys, the more severe the disease the more material passed through the capillary walls. Nauwerck, 1886, Hansemann, 1887, and Engel, 1901, believed the increased permeability was due to the degeneration and desquamation of the glomerular epithelium.

Changes in the Glomerular Basement Membrane: Ohmori, 1921, was the only writer to note changes in the basement membrane in nephritis. He believed it could be mechanically compressed and physically or chemically altered. He observed that it was often attached to the intracapillary hyaline masses seen in old chronic glomerulonephritis. Within the loops fibrin was deposited on the membrane and finally fused with it.

Changes in the Vas Afferens: Langhans, 1879, Nauwerck, 1886, Reichel, 1905, Löhlein, 1910, Mallory, 1914, MacCallum, 1916, Herxheimer, 1918, Gross, 1919, Aschoff, 1921, Bell and Hartzell, 1922, and Fahr, 1925, have all concluded that glomerulonephritis is a primary inflammation in the capillaries of the glomeruli. Other workers have believed the primary change to take place in the vas afferens with a secondary inflammation of the glomeruli. Klein, 1877, after finding a multiplication of muscle and connective tissue nuclei in the coat of the afferent vessel, believed the thickening of

its wall tended to shut off the circulation to the glomeruli. Hlava and Thomayer, 1882, were of the opinion that a small round-celled infiltration in the interstitial tissue around the afferent and efferent vessels compressed their lumina. Aufrecht, 1886 and 1894, confirmed the findings of Klein. Herxheimer, 1918, felt that Aufrecht's conclusions were due to the fact that he was not studying the earliest stages of the disease. Volhard and Fahr, 1914, decided that a spasm of the afferent vessel led to ischemia of the glomerulus and secondary inflammatory processes there. Kuczynski and Dosquet, 1926, described a severe edema in the region of the afferent vessel with occlusion of its lumen in early acute nephritis. The edema stimulated resting cells of the adjacent connective tissue until they wandered into the wall of the vas and formed new cells around the hilum. Hückel, 1928, disagreed with Kuczynski and Dosquet after studying a very early acute case, a woman aged 39 years, who died of uremia thirty hours after the onset of symptoms. He was unable to find swelling or edema at the vascular hilum in more than a very few glomeruli. All workers have agreed that there is a great deal of arteriolar disease in the advanced stages of glomerulonephritis.

Hyalinization of the Glomerulus: Klebs, 1863, concluded that the connective tissue cells between the loops as well as emigrated leucocytes changed into a hyaline substance which later became organized. Klein, 1877, believed there was a primary arteriolar lesion which led to a hyaline degeneration and fibrosis of the glomerulus and its capsule. Thoma, 1877, described a connective tissue change of the vascular loops of the glomerulus. The walls thickened and acquired a homogeneous appearance while the capsule formed concentric layers of fibrous cellular connective tissue. In one type of atrophy the loops did not completely disappear while in the other type they were replaced by concentric layers of connective tissue. Ziegler, 1879, described two types of atrophy, one due to the proliferation and pressure of a crescent on the tuft, the other due to obliteration of the vas afferens. Greenfield, 1880, believed proliferation of glomerular connective tissue cells as well as the crescent led to fibrosis of the glomerulus. Waller, 1880, concluded that the hyalinization of the malpighian body was due to a connective tissue transformation of leucocytes which had migrated into the capsular space. The connective tissue cells normally present between the loops proliferated and assisted in the process. Dunin, 1883, found

three types of contracted glomeruli due to Bright's disease. The types differed in size, fusion or non-fusion of glomerulus and capsule, and partial or total loss of the original lobular structure. Friedländer, 1883, believed the walls of the capillary loops thickened until the lumina were occluded. Nauwerck, 1886, described a hyaline thickening (similar to amyloid degeneration) of the loop walls, plus an occlusion of the capillaries by leucocytes, endothelium and granular masses. Böhm, 1897, observed pericapsular and hilum connective tissue proliferation causing atrophy of glomeruli. Goemans, 1899, described a connective tissue proliferation in the glomerulus and in the capsule. Engel, 1901, believed that organization of the fibrin in the exudate was the source of the connective tissue growth of loops and capsule. Tschistowitsch, 1903, saw pericapsular connective tissue growing in through the capsule at various points and also from the hilum. In some cases swelling of the capsular basement membrane and collapse of the capillaries were the sources of the capsular and glomerular hyalin. Löhlein, 1910, described a homogeneous thickening and hyalinization of the loop walls and fusion of the whole with the capsule. Mallory, 1914, found that a proliferation of fibroblasts in the tuft produced more collagen which contracted toward the hilum until the capillaries were obliterated. This left a non-vascular mass of hyaline connective tissue. Krauspe, 1922, considered glomerular hyaline to be different chemically from capsular hyalin, as shown by their reactions to the Van Gieson stain. He concluded that the two hyaline materials were in a different colloid state. Calcification of the hyaline glomerulus has been reported by several authors.

Summary

1. The opinion has been quite uniformly held that there is a marked proliferation of glomerular endothelium in the acute stages of nephritis.

2. The leucocytic exudate has usually been described as polymorphonuclear. The origin of the mononuclear cells within the capillaries is doubtful, whether from the blood stream or from freed endothelium.

3. Protoplasmic, albuminous and fibrinous types of intracapillary network have been reported. There has been no agreement on this point.

4. Proliferation, degeneration and desquamation of glomerular epithelium have been established as one part of the morphological change.

5. There has been no uniform explanation of the origin and organization of the cells of the crescent.

6. Changes in the vas afferens have been considered for the most part as secondary to the inflammation in the glomerulus.

7. Very little attention has been directed toward alterations in the glomerular basement membrane.

8. The process of hyalinization of glomeruli in nephritis has not been described in detail.

II. Results Obtained from a Microscopic Study of Sixty Cases of Clinical Glomerulonephritis

Sections were stained as described in the previous paper. The descriptions below apply to the Mallory–Heidenhain azan-carmine stain unless otherwise specified.

Glomerular Epithelium: These cells covering the outside of the loops are among the first to react in nephritis. There is a cytoplasmic increase up to two or three times the original amount. With the azan-carmine it appears pale purple and denser than the capsular epithelium. With Mallory's eosin-methylene blue stain the periphery of each cell is basophilic, while the center is acidophilic. The nuclei enlarge and often become indented as if preparing for amitosis. Many cells contain two identical nuclei lying side by side. Mitosis has not been observed. The nuclear membrane is very delicate, and except for one to three nucleoli the chromatin is diffusely distributed in very minute particles. The nuclei are always situated in the periphery of the cell as far from the glomerular basement membrane as possible. Only a thin rim of cytoplasm separates the nucleus from the capsular space. Whether by mitotic or amitotic division, these cells are soon increased to two or three times their original number. This swelling and proliferation occur around every loop in every glomerulus and completely fill in the space normally present between lobules. When cut through the hilum and the junction with the convoluted tubule, the glomerulus appears oval in shape. Often one lobule with its enormous epithelial cells projects into and almost occludes the tubule.

The next change in the epithelium seems to be degenerative. Small hyaline granules and vacuoles appear in the cytoplasm of some of the cells. These bodies stain deep blue and are similar to the hyaline granules so commonly observed in the convoluted tubules in amyloid disease. At the same time the attachment of many of the cells to the loop wall becomes so narrow that it resembles a pedicle (see Fig. 3). This keeps the bodies suspended in the capsular space. The cells are so numerous that in spite of the narrow pedicles the loops are not left bare.

The hyaline granular degeneration may increase until the entire cell is filled. The nucleus may or may not disintegrate. In any case a certain number of cells are desquamated. They lie in the capsular space between fibrin clumps or applied to the capsular epithelium or on the inner surface of a crescent. When the pedunculated shape is retained the glomerular origin is evident, and even when the cell rounds off, its cytoplasm is much denser and more darkly staining than that of the capsular epithelium.

Desquamation is not extensive and soon ceases. Hyaline granular and vacuolar degeneration may continue for some time. As the intracapillary lesions progress, the swelling and proliferation of the glomerular epithelium either stay at a standstill or regress. The cases showing the most desquamation are left with very little epithelium. Bare loops have not been observed.

There is a reason for believing that the degeneration follows proliferation. It is the observation that the earliest intracapillary changes are accompanied by proliferation of epithelium, while the more advanced intracapillary changes seem to be accompanied by degeneration and desquamation.

Glomerular Basement Membrane: During the early stages of glomerulonephritis the changes in the glomerular membrane are rather insignificant. In those glomeruli showing extensive hemorrhage into the capsular space there is much wrinkling and indentation of the membrane, probably due to the external pressure. A careful search was made for pores or tears in the membrane through which the red and white blood corpuscles might have escaped. No openings were found even when the extravasation was so recent that it was not hemolyzed.

Glomerular Endothelium: The endothelial cells lie inside the glomerular basement membrane. They react by swelling and prolifera-

tion so that very early in nephritis there is a complete cytoplasmic wall lining the loops. As many as five mitoses have been seen in one section of a glomerulus. Multiplication continues until there are two or three layers of these cells in each loop (see Fig. 2). The nuclei are now very large and usually elongated due to crowding. Many are deeply indented, the chromatin is coarse, and there may be nucleoli. There is a large amount of cytoplasm, but the outline of each cell is irregular and rather frayed as if it had recently been attached. It appears as if many are desquamated into the lumen, where they are held in a meshwork of leucocytes, fibrin, and hyaline fibers (to be described later). At least in favor of desquamation is the fact that these large mononuclear cells seen in the lumen of the loop are identical morphologically with the adjacent endothelium.

Occasionally pyknosis and karyorrhexis are seen in endothelial cells. This condition is difficult to distinguish from the disintegrating polymorphonuclears, which always seem to stick to the capillary wall.

Glomerular Intracapillary Leucocytes: Polymorphonuclears are not as conspicuous in this series of cases as in those of other authors. In the very early acute cases there are from one to two in each loop, that is, they are about one-third to one-half as numerous as the endothelial cells. When pyknotic they are seen adhering to the capillary wall, while when healthy they remain out in the lumen between the mononuclears. A few eosinophils but no lymphocytes have been observed.

The lumina of the glomerular capillaries are occluded for the most part by mononuclear cells. Some of these may be monocytes. With the stains used in this study neither their nuclei nor their cytoplasm could be distinguished from that of the proliferating endothelium. The numerous mitoses, the adjacent position of these cells and their similar morphology, all point to their endothelial origin. They occasionally appear to be free cells, but usually lie in layers or in a mass. Sometimes the lumen is completely stopped up with cells, but usually there is a narrow slit toward one side of the loop where the circulation may continue.

Neither polymorphonuclears nor mononuclears were ever seen passing through the glomerular membrane. There was no evidence of phagocytosis of disintegrating cell fragments.

Intracapillary Fibrin and Débris: Fibrin in clumps, rods or threads

is occasionally seen in a few glomerular loops. It usually lies close to the capillary wall and takes a bright red color with the Mallory–Heidenhain technique. Coagulated albumin, disintegrating red blood cells and pyknotic polymorphonuclears may be found in the lumina of the loops.

Intracapillary Hyaline Fibers: (Figs. 2 and 3.) It is these hitherto undescribed fibers which eventually lead to hyalinization of the glomerulus. It is believed that they are a typical finding in clinical glomerulonephritis. They were found in all but two of our sixty cases.

These hyaline fibers are present in the earliest stages as fine lines lying between the proliferating endothelial cells and leucocytes. Knots are numerous at intersection points. The fibers with their knots stain deep blue (exactly the color of the glomerular basement membrane) with Mallory–Heidenhain, red with Van Gieson, not at all with silver and are practically invisible with Mallory's eosin-methylene blue and with hematoxylin-eosin. They seem to be attached at one end to the glomerular basement membrane. They always lie along the edge of the cytoplasm of the intracapillary cells. The fibers form a network within the capillary which helps to hold in place the leucocytes and endothelial cells. The occurrence of the fibers is diffuse in most of the loops of almost all glomeruli. They are not seen except in association with endothelial proliferation. Therefore, they are inconspicuous in (1) those cases which show only crescent formation with very little intracapillary lesion and (2) in those glomeruli which are compressed by the hemorrhage in the capsular space.

As the disease progresses, these fibers increase in diameter and in number until they form a dense meshwork enclosing the newly formed endothelial cells. In the earlier stages a few pyknotic polymorphonuclears may be included, but as the fiber meshwork thickens only mononuclear cells remain. The polymorphonuclears have disintegrated. The fibers remain attached to the basement membrane in many places. Usually a narrow channel is left along one side of the loop where the circulation continues. By this time the fibers are much thicker in caliber than the basement membrane of the loop they occupy (Figs. 4 and 5).

The origin of the hyaline fibers is not as yet determined. The possibilities would seem to be: (1) that they are branches or offshoots

from the glomerular basement membrane; (2) that they are fibrin threads which become transformed into collagen; (3) that they are products of disintegrated red or white blood cells; and (4) that they are reticulum or collagen fibers formed intercellularly from the constituents of the blood stream under the influence of a secretion from the proliferating endothelium. In this latter case the disintegrating polymorphonuclears might have some influence.

It should be mentioned that these fibers are believed to be real and not artefacts, because they have not been seen in the sections of numerous normal control kidneys.

The points in favor of the first possibility are the facts that the fibers and membrane are usually attached to one another and that their staining reactions are similar. The demonstration of a fibrillar character in the membrane would give support to this theory.

The work of Baitsell, 1915, 1916, 1917, and 1921, would point toward the feasibility of the second theory. He has shown in living cultures and in wound healing the transformation of a fibrin net into fibrous tissue either by mechanical factors alone or by the influence of living cells. Foot, 1921, believed collagen fibers were a product of the transformation of fibrin, but in 1925 concluded that this was erroneous and that the two were independent of each other. He disagreed with White and Smith, 1924, who believed that fibrin in the center of a tubercle was converted to reticulum by the agency of endothelium. Foot, 1925, demonstrated a reticular network in tubercles one week old before the fibrin appeared. He believes fibrin plays no part in the formation of reticulum and that reticulum is converted directly into collagen. However, the fibers in glomerulonephritis stain blue, while the fibrin is red and both are present at the same time. When first formed, the hyaline fibers are fine sharp lines while the fibrin is usually in coarser rods or clumps. If the fibers do come from fibrin, the colloid state of the latter must be considerably altered and with that its staining reaction and morphology.

With regard to the third possibility, it has been shown by Foot, 1927, that reticulum fibrils in hanging-drop tissue cultures of blood can come from the disintegration of erythrocytes and a rearrangement of the reticulum they contain. He does not suggest that this occurs frequently in the body but that other cells might have an intracellular network which would be deposited by lysis. That red blood cells are not the origin of the intracapillary fibers in glomerulo-

nephritis can be shown with silver impregnation. The disintegrating red cells and their fragments in the convoluted tubules take the silver stain, while in the same section the intracapillary fibers do not.

The fourth possible origin of the fibers involves a discussion of the relation between reticulum and collagen. Russakoff, 1908, first described the transformation of reticulum into collagen. Miller, 1924 and 1927, considered reticulum as precollagen, for in tubercles where there was a tendency to heal, the former became converted into the latter. Baitsell and Mason, 1926, and Baitsell, 1927, have also made a study of the origin of fibrous tissue in tuberculous inflammation. They believe there is a transformation of the intercellular exudate into fibrils and fibrous tissue. "This does not preclude the possibility that the movement of the cells through the exudate may not, by means of purely mechanical factors, change the structure of the fibrous material, or that the secretions of various types of cells may so influence the fibers that their chemical composition becomes changed." The very detailed work of Orsós, 1926, shows that reticulum is only changed collagen and transitions in either direction from one to the other can be seen in normal lymph nodes. He states, and his illustrations show that "excluding certain pathological conditions" reticulum or collagen formation is intracellular. This substance is first seen inside the cytoplasm of the cell as blue-staining elements with Mallory's anilin blue. However, the intracapillary fibers in glomerulonephritis with this same stain seem to be intercellular rather than intracellular. Mallory and Parker, 1927, in a study of reticulum and collagen, concluded that all collagen is produced by fibroblasts, that endothelial cells do not produce an intercellular substance and that reticulum is collagen in a separated form. In order to fit into this theory the intracapillary mononuclear cells in nephritis would have to be considered fibroblasts. Certainly there are no connective tissue cells out in the tips of the loops according to any authority. Yet fiber production is just as plentiful there. According to this study there are only endothelial cells inside the basement membrane of a loop. Therefore the fibroblasts would have to come from endothelium. But the new cells are morphologically more like endothelium than like fibroblasts. The nuclear membrane is thick and often indented, and the chromatin is arranged in rather coarse clumps. These cells do seem to assist in the formation of fibers which ultimately are indistinguishable from collagen. Per-

haps endothelium and its derivatives can produce fibers. Maximow, 1928, studied the formation of reticulum and collagen in cultures of connective tissue and also of blood leucocytes. The process is to be thought of as a crystallization or precipitation. The colloidal sol surrounding the fibroblasts is changed into a gel under the influence of a chemical excretion from the cell bodies. Fiber formation is intercellular. The fibers first appear in the form of an argyrophil network, which is identical with reticulum. With increase in amount, they become parallel and arrange themselves in wavy bundles. At this stage they lose their argyrophil properties and stain blue with anilin blue or red with Van Gieson. Often the fibrin threads form a supporting framework for the fibers, but no direct transformation from one to the other was observed. Above all, the fibers develop extracellularly. The intracapillary glomerular fibers are similar to Maximow's in that they form extracellularly and perhaps from the constituents of the blood. Maximow would explain the newly formed endothelial cells as fibroblasts. On the other hand, the hyaline fibers do not stain with silver even in the early stages but seem to be closely allied to collagen from the beginning.

Thus far in this study it would seem that the hyaline intracapillary fibers in nephritis either come from the glomerular basement membrane or arise intercellularly in a manner comparable to those described by Miller, 1924 and 1927, Baitsell and Mason, 1926, Baitsell, 1927, Mallory and Parker, 1927, and Maximow, 1928. If they arise from the constituents of the blood stream, it must take place under the influence of the proliferating endothelium and disintegrating leucocytes. The fact that the fibers do not stain with silver may be due to lack of sufficiently early stages of nephritis to study.

Glomerular Connective Tissue: As the glomerulus increases in size in the early stages of nephritis, it extends farther down on the vas afferens. Thus more connective tissue cells appear to follow the afferent vessel into the glomerulus. In addition to this apparent increase there is a slight proliferation of these cells. Their location prevents confusion with the intracapillary cellular proliferation. In no case was there seen sufficient proliferation of these cells to occlude the lumen of the afferent vessel. There is a marked increase in reticulum fibrils in contracted glomeruli.

The Capsular Basement Membrane: In the early stages it shows no change. Many authors have described a thickening where it turns

to be reflected over the glomerulus. This was not observed in any of the sixty cases. In later stages the reticular framework thickens and forms multiple concentric layers outside the inner apparently homogeneous basement membrane.

Capsular Epithelium: These cells react similarly to glomerular epithelium. In the early periods of the disease they become swollen, show mitoses and multiply rapidly. They often show hyaline granular degeneration. The intracellular fibrin clumps described by some authors seem on the contrary to be extracellular. The epithelium may form a very cellular single layer or multiple layers.

Crescent Formation: In the cases studied crescents were formed chiefly from proliferating capsular epithelium. When there is marked glomerular epithelial desquamation, the freed cells line the inner surface and may form the inner layers of the crescent. They may be distinguished from the capsular epithelium by their greater size, irregular shape, pedicles and dense cytoplasm. The regularly arranged outer portion of the crescent forms from capsular epithelium. Fibrin lies in threads, sheets and clumps between the cells. It appears bright red with the Mallory–Heidenhain stain. Blue hyaline fibers are also found in threads between the cells. These are very similar to the intracapillary fibers previously described. They have both knobs and knots at intersections. They soon form a meshwork in which the epithelium lies. This often occludes the convoluted tubule. Tubule-like cavities may appear in the crescent. The fibrin, polymorphonuclears and disintegrating desquamated glomerular epithelium soon disappear. The hyaline fibers seem to fuse with the capsular basement membrane. When adhesions develop between crescent and glomerulus, the hyaline fibers of the crescent are continuous with the glomerular basement membrane and through it with the intracapillary fibers. The closely related mesenchymal origin of all the cells in a glomerulus makes it theoretically possible for any one cell to produce fibrils. The origin of the fibers in the crescent is not yet determined. In many areas they seem to come from the capsular basement membrane, while in other places they appear to be forming intercellularly without attachment.

Hyalinization of the Glomerulus: The intracapillary hyaline fibers increase in number and caliber until they form the bulk of the intracapillary glomerular masses. The enclosed mononuclear cells are not allowed much space for cytoplasm. Sometimes the whole loop

is filled with the mass of fibers and cells all the way out to its basement membrane. This allows no lumen for the circulation. When all the loops in a glomerulus are as extensively involved, the nuclei are gradually squeezed out of existence; then adjacent loops fuse. At the same time, if crescents are present the hyaline fibers there increase and press on the enclosed cells until they too disappear. The capsular basement membrane, crescent, glomerular basement membrane and loop contents all fuse. There is a general contraction, and more nuclei disappear. Finally only a hyaline fibrous mass with very few nuclei remains. Even in this end stage it is usually possible with the Mallory–Heidenhain stain to differentiate the capsular and crescent fibers from those of glomerular origin.

Summary

From a microscopic study of glomeruli of sixty cases of acute and chronic glomerulonephritis the conclusions are as follows:

1. The intracapillary cellular increase is chiefly endothelial and is explained by the numerous mitoses. The leucocytes are polymorphonuclear and are much less numerous than the endothelial cells. A small amount of fibrin and débris is found in most of the capillaries.

2. The extracapillary changes consist of proliferation, degeneration and desquamation of glomerular and capsular epithelium. These are easily distinguished from intracapillary lesions because the glomerular basement membrane lies between.

3. Crescent formation may be due to capsular epithelial proliferation or to a combination of capsular proliferation and glomerular epithelial desquamation. In a very early period hyaline fibers appear between the cells and are soon continuous with the capsular basement membrane.

4. Hyaline fibers also appear inside the glomerular capillaries. They stain blue with the Mallory–Heidenhain azan-carmine, or with Ohmori's rein blau and not at all with silver. When first formed, they lie between the proliferating endothelial cells and are usually attached to the basement membrane. They appear as fine sharp lines with knobs at intersections. They soon form a definite meshwork enclosing the intracapillary cells. This fibrous meshwork is be-

lieved to be an important and typical finding in glomerulonephritis. It is the initial stage of the hyalinization of the glomerulus. The process takes place in all the glomeruli but not always to the same degree. When the loops are only partially occluded, blood continues to circulate along one side and the glomerulus has some function. When every loop is occluded, the circulation cannot continue. The fibers within the loop increase in number and caliber, many fuse, and the enclosed cells are atrophied by pressure. The glomerular loop arrives at a stage where it consists of a few flattened epithelial cells, a basement membrane and a lumen completely filled with a hyaline fibrous mass enclosing a few nuclei. Adjacent loops fuse, and the fibers contract. The glomerular epithelium atrophies and disappears. At the same time the fibers of the crescent have increased and contracted until they have eliminated most of the cells. The fibrous crescent now fuses with the fibrous glomerulus. The end result is a sphere of fibrous connective tissue containing practically no nuclei.

5. The origin and composition of the intracapillary hyaline fibers are undetermined and require further study. They do not seem to be fibrin or derivatives of it. They are thought to be a crystallization or precipitation of the intercellular exudate under the influence of the proliferating endothelial cells or perhaps a product of the glomerular basement membrane. Later they are indistinguishable from typical collagenous fibers.

6. The above processes are typical of clinical glomerulonephritis. The finding of a glomerular cellular increase (usually monocytes) following pneumonia and other infections is not to be confused with clinical nephritis. The non-clinical cases do not have the characteristic intracapillary fibrous meshwork.

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DESCRIPTION OF PLATES

PLATE 105

FIG. 1. One loop of a normal glomerulus. Gl. ep. = glomerular epithelium; gl. b. m. = glomerular basement membrane; end. = endothelium; r. b. c. = red blood cell; l. = leucocyte.

FIG. 2. One loop of a glomerulus from a case of acute glomerulonephritis. Note the swollen, glomerular epithelium, the large endothelial cells in several layers and the intracapillary hyaline fibers. C. b. m. = capsular basement membrane; c. ep. = capsular epithelium; gl. ep. = glomerular epithelium; gl. b. m. = glomerular basement membrane; end. = endothelium; end. 2. = proliferating endothelium; hy. f. = hyaline intracapillary fibers.

FIG. 3. One loop of a glomerulus from a later stage of acute glomerulonephritis. Gl. ep. = glomerular epithelium; gl. b. m. = glomerular basement membrane; end. = endothelium; end. 2 = proliferated endothelium; hy. f. = intracapillary hyaline fibers.

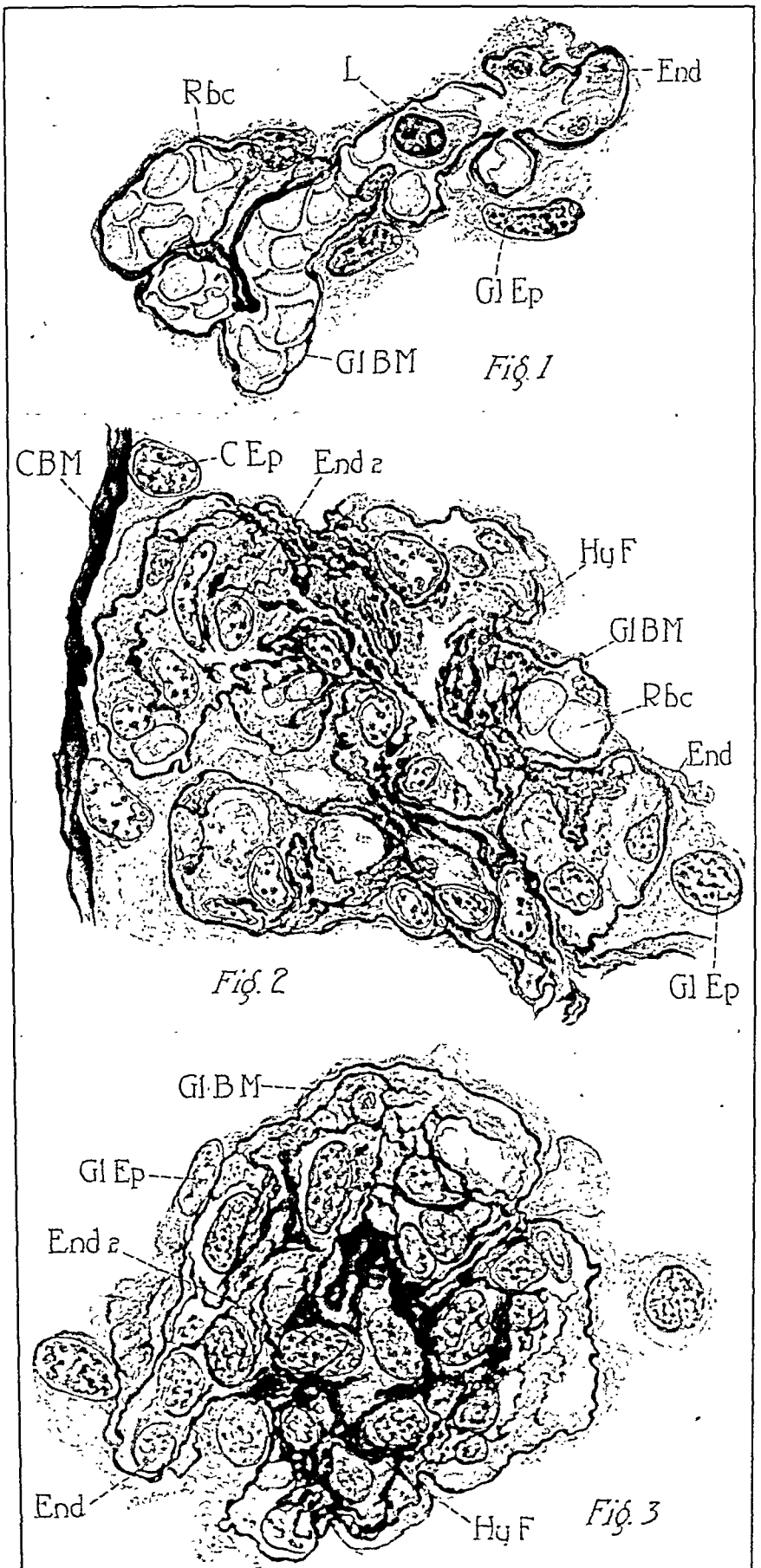

L
End
Rbc
Gl Ep
Gl BM
Fig. 1
CBM
C Ep
End
Hy F
Gl BM
Rbc
End
Gl Ep
Fig. 2
Gl BM
Gl Ep
End
End
Hy F
Fig. 3

PLATE 106

FIG. 4. One loop from a glomerulus of chronic glomerulonephritis. Gl. ep. = glomerular epithelium; gl. b. m. = glomerular basement membrane; end. = endothelium; end. 2 = proliferated endothelium; r. b. c. = red blood cell; hy. f. = hyaline fibers.

FIG. 5. One loop from a glomerulus of chronic glomerulonephritis. C. b. m. = capsular basement membrane; gl. ep. = glomerular epithelium; end. = endothelium; end. 2 = proliferated endothelium; hy. f. = intracapillary hyaline fibers.

Figures 1, 2, 3, 4 and 5 are camera lucida drawings from oil immersion fields.

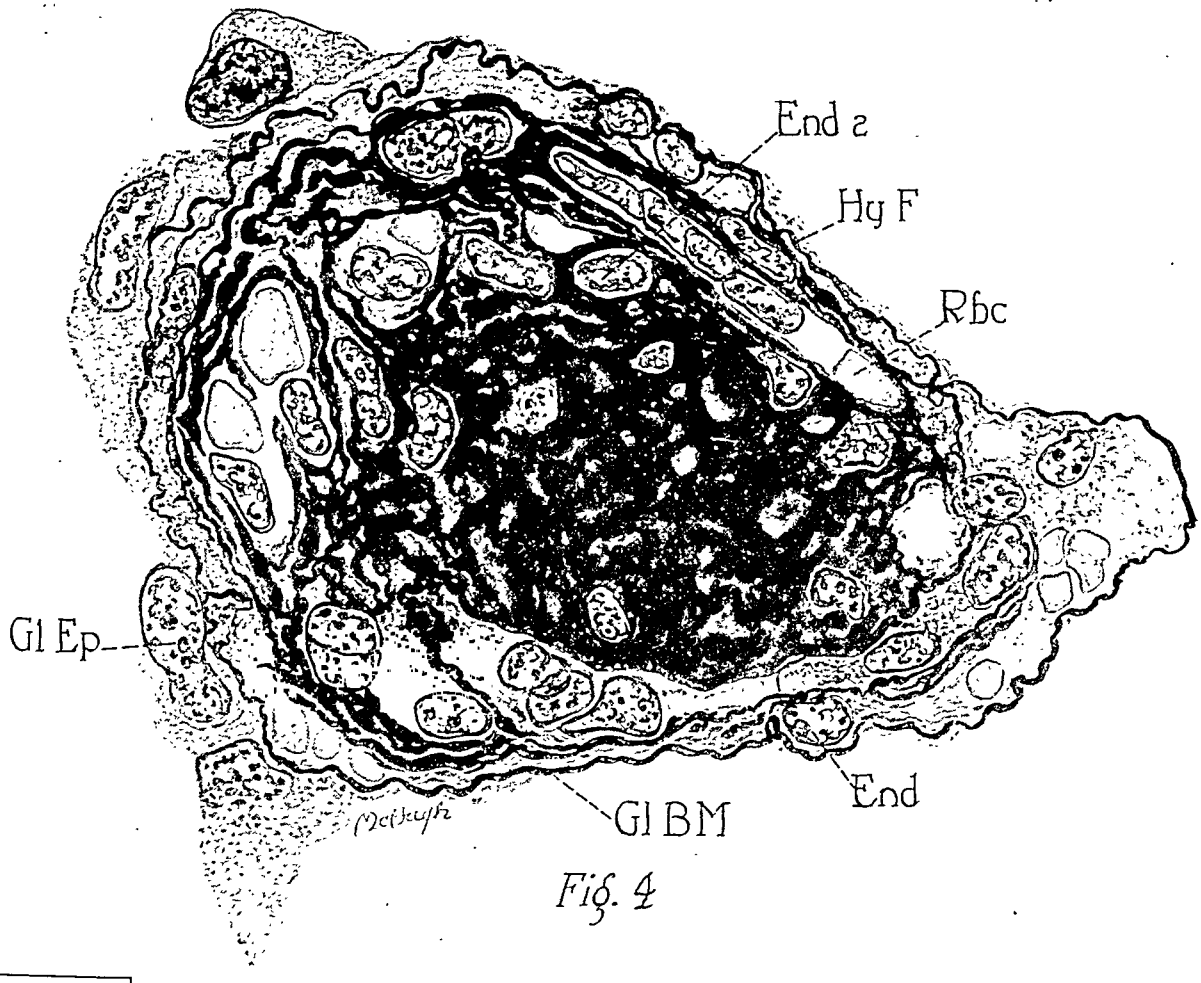


Fig. 4

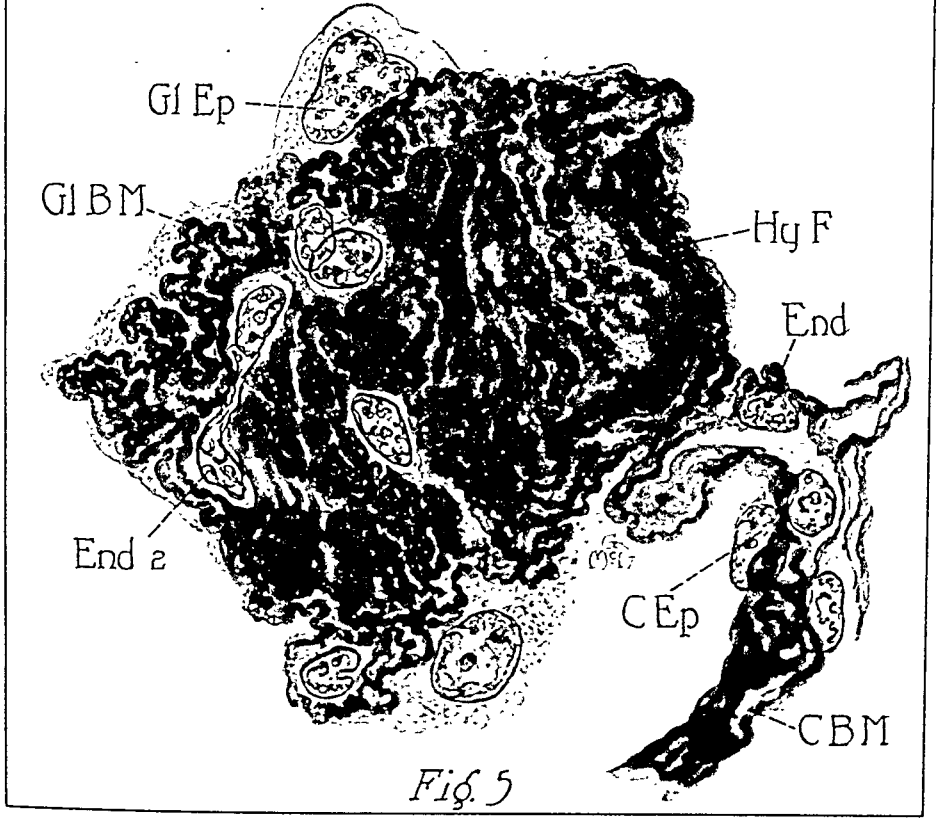


Fig. 5

LIPOID NEPHROSIS *

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Nephrosis is defined as a degenerative renal disease in which the lesions are restricted chiefly to the tubules. On the basis of etiology several types are distinguished:

1. Nephroses due to simple chemical poisons (*e. g.*, corrosive sublimate).
2. Nephroses due to poisons of metabolic origin (*e. g.*, bile acids).
3. Nephroses due to poisons of bacterial origin, as in pneumonia, diphtheria, abscess, etc.
4. Nephroses due to the toxemia of pregnancy.
5. Amyloid nephrosis.
6. Lipoid nephrosis.

This paper deals only with lipoid nephrosis — a type which has attracted much attention in recent years. The study has been directed chiefly toward the structural changes in the kidneys and the anatomical and clinical distinctions between lipoid nephrosis and glomerulonephritis. Some of the questions that have arisen in connection with the problem of lipoid nephrosis are: (1) Is lipoid nephrosis a renal disease or a general metabolic disorder? (2) Is it a distinct type of renal disease or merely a form of glomerulonephritis? (3) What is the nature of the mixed type — nephritis with nephrotic *Einschlag?* (4) Are all the non-contracted kidneys, associated with albuminuria and edema, instances of lipoid nephrosis? (5) Are there any certain clinical or anatomical distinctions between lipoid nephrosis and glomerulonephritis?

The authors chiefly responsible for the wide recognition of lipoid nephrosis as a distinct disease are Munk, Volhard and Fahr, and Epstein. Munk described it under the name of "lipoid nephrosis"; Volhard and Fahr, as "genuine nephrosis"; and Epstein, as "chronic nephrosis." The term suggested by Munk has been generally adopted since it refers to a striking characteristic of the disease.

* Received for publication June 24, 1929.

Lipoid nephrosis was defined anatomically by Volhard and Fahr under the designation of "genuine nephrosis." Nearly all subsequent contributors to this subject have accepted the criteria laid down by these authors. They described the kidneys as enlarged with smooth external surfaces and thickened cortices of yellowish color. The glomeruli appear normal or show only minor focal lesions. Large numbers of lipoid droplets are found in the convoluted tubules, and some may be seen in the interstitial cells and in the glomeruli. There is no tubular atrophy.

Clinically there is general agreement that the outstanding features are the presence of severe albuminuria and marked edema, and the absence of hypertension, cardiac hypertrophy, hematuria and uremia. There is a definite hypercholesterolemia and a decrease of the plasma proteins with reversal of the normal albumin:globulin ratio. Oliguria and decreased elimination of chlorides are frequently noted. The urine commonly contains doubly refractive droplets of lipoid (lipoiduria).

It is not known whether all kidneys with lipoid infiltration of the tubules are associated with the clinical phenomena of lipoid nephrosis. In the present state of our knowledge the diagnosis should not be made on the anatomical findings alone.

Some cases of chronic glomerulonephritis in the earlier stages present the clinical features of lipoid nephrosis, so that a diagnosis based on clinical data alone may be erroneous.

Frequently a renal disease is seen that presents all the clinical features of lipoid nephrosis except for the presence of hypertension or definite nitrogen retention. Epstein included cases of this type in his group of "chronic nephroses"; but they are usually considered a mixed form of nephritis and nephrosis. Fahr refers to this group as nephritis with nephrotic *Einschlag*, which may be translated as nephritis with a nephrotic tendency. The structural changes in the kidneys in this type will be described later.

Table I gives a résumé of all the available published reports in which the diagnosis was established by postmortem examination. The anatomical findings in these cases correspond with Volhard and Fahr's definition.

Table II gives a résumé of the published reports of living persons in which the clinical data seem to justify the diagnosis of lipoid nephrosis. Cases with high blood pressure have not been tabulated

since these are presumably not pure nephroses. When the blood pressure is not recorded the case is excluded because its exact nature is uncertain. Nephroses associated with chronic suppuration have also been omitted since they are presumably of the amyloid type.

In addition to the cases shown in the tables there are reports dealing with groups of patients without individual details. Clausen observed 23 cases of parenchymatous nephritis (nephroses) in children. There were ten deaths. Infection was the chief cause of death. In the single postmortem that was performed the glomeruli showed only a few polymorphonuclear leucocytes.

Schwarz and Kohn observed 17 cases of nephrosis in children. The diagnostic features were albuminuria and edema with hypercholesterolemia and reduced plasma proteins. Blood pressure and renal function are not mentioned. No postmortems were reported.

Bannick and Keith reported 25 chronic nephroses. Their criteria were: edema for several months without demonstrable vascular disease, a blood pressure not above 140/90, blood urea not above 40 mg. per 100 cc., hypercholesterolemia, decreased plasma proteins, no hematuria, no retinitis. There were two deaths but no postmortem reports were given.

Munk, in 1913, reported 5 cases of lipoid nephrosis with albuminuria, edema, lipoiduria and oliguria. Blood pressure was given in only one instance. Four of the patients had syphilis.

Among 300 cases of nephritis with edema, Schlayer found only 6 cases of pure lipoid nephrosis and some of these were uncertain.

Before discussing the several phenomena of lipoid nephrosis in detail, I shall give the results of my own studies on this subject.

Group I. Pure Lipoid Nephrosis

Case I. *Clinical History:* A–28–647. Male, 28 yrs. old. The patient stated that he developed a cold about April 3, 1928, and one week later noticed swelling of the legs and puffiness about the eyes. After a few days the swelling became more prominent and involved the skin of the chest and back. The only subjective symptom was weakness. The urine was dark brown in color and reduced in amount. He was admitted to the hospital April 17, complaining of weakness and edema. Physical findings were negative except for severe generalized edema and moderate tenderness over both kidney regions posteriorly. Hemoglobin was 97 per cent on admission and 81 per cent two weeks later. Erythrocytes were 4,970,000 on admission and 4,600,000 later. Leucocyte count April 17, 7150 with 66 per cent polymorphonuclears. There was a definite oliguria. The specific gravity of the urine was constantly high, 1021 to

TABLE I

Lipoid Nephroses in which the Diagnosis was Confirmed by Postmortem Study

Author	Age in yrs.	Sex	Duration	Outcome	Edema	Urine albumin gm. per 1000 cc.	Cholesterol mg. per 100 cc.	Serum albumin	Serum globulin	Total protein	Blood pressure	Urea nitrogen mg. per 100 cc.	N.P. nitrogen mg. per 100 cc.	Phenolsulphonephthalein	Urine amount in cc.	Wassermann	Basal metabolic rate	Peritonitis	Weight of kidneys in gm.	Lipiduria
McElroy.....	17	M	2 mo.	D	3	4					115–75		77		360	—		+		
Kaufmann, Mason .	29	M	7 mo.	D	3	5 to 8		Normal ratio			142–98 122–90	24 39		48	2030 0	—		+ +	570	
Bock, Mayer.....	43	F	3 mo.	D	3	12					95– 100–85				1700 500			+ 		
Rigler, Rypins.....	2½	M	9 wk.	D	3	+++	366	3.08	3.39	6.47	100–80	10.3 20.0								
Murphy, Warfield..	21	M	1 yr. +	D	3	++	500 700				118–70 125–65	Normal		60		— —	—10 —15		415	+ +
Fahr.....	19	M	6 wk.	D	3	16 to 60					120–		31							+
	38	F	10 mo.	D	+	22 to 60					132–		34		less					
	9	M	2½ mo.	D	3	15 to 25					108–							+		
	18	M	4 wk.	D	3	12 to 30					128–				less					
	7	M	9½ mo.	D	+	+									less					
	16	F	15 mo.	D	+													+		

Volhard, Fahr.....	15	M	15 mo.	D	+	5 to 51					120–58				200 2600			+		
	22	M	6 mo.	D	2	2 to 14					130–90		29 75		550 800			+	510	
Stepp, Petri.....	16	M	6 mo.	D	3	24	960				110– 120–		46		200					
Vandorfy.....	24	M	7 mo.	D	2	3 to 24					135–				800			+	large	
Stolz.....	3	M	1 mo.	D	3	6					80–							+	large	
	51	M	2 yr.	D	1,3	5					120– 105–75							+	large	–
Major, Helwig.....	34	M	10 mo.	D	3	+++		33%	66%		140–90	15		22	less		–31		430	
Löwenthal.....	8	M	2½ yr.	D	3	2 to 28					88–58 120–90	Normal			180 1450					+
	4½	F	17 mo.	D	3	0.5 to 20	400 770		50%	6.5	Normal				100 500					
	29	F	3 mo.	D	3	3 to 28	300				Normal				100 800	–				+
	21	M	4 mo.	D	3	1 to 45	420			3.9	Normal	Normal			300 2600	–			large	+
	16	F	14 mo.	D	3	8 to 12	306				Normal				300 800	–				+

TABLE II

Lipoid Nephroses in which the Diagnosis is Based on Clinical Data

Author	Age in yrs.	Sex	Duration	Outcome	Edema	Urine albumin gm. per 1000 cc.	Cholesterol mg. per 100 cc.	Serum albumin	Serum globulin	Total protein	Blood pressure	Urea nitrogen mg. per 100 cc.	N. P. nitrogen mg. per 100 cc.	Phenolsulphonephthalein	Urine amount in cc.	Wassermann	Basal metabolic rate	Lipoiduria
Campanacci.....	3			Rec.	3	8					Normal							+
	48	F	3 yr.	Rec. alb. +	3	3 to 55					130–		42		600 800	–		+
	18	M		Rec.	2	5 to 6					120– 125–				500 600			±
Epstein, Lande ..	14	F	9 mo.	Imp. alb. +	3	+++	904				125–70	19.6		30			–18	
	27	F	2 yr. (?)	Imp. edema	3	+++	364				110–70	22.4		45		+	–15	
	33	F	7 mo.	Imp. alb. +	2	+++	375				95–60	14		35			–18	
	29	F	5 mo.	Recur	2	+++	508				110–70	18.2		30			–8	
	35	M	12 yr.	Imp. alb. +++	+++ to 0	+++	320			4.3	105–68	15.4					–15	
Rigler, Rypins...	21	F	8 mo.	Imp.	++	+++	330	2.19	5.03	7.2	115–65	9.8 16.0		55		–	–25	
	34	M	13 mo.		+++	+++	Milky	2.7	3.4	6.1	132–84	9.6 33.8		52		–	–12	

Bennett.....	22	M	18 mo.	Imp. alb. +	++	+++	117 528				140–90 100–70	Norm.				−	−19	
Grossman.....	27	M	6 mo.	Rec. alb. +	+++	12					120–		44		500 600			+
Murphy, Warfield	25	F	4 yr.	Not rec.	+++ to 0	++	266 388				128–80	16.8		65				+
	24	M	3 mo.	Not rec.	++ to 0		666 275				110–60 130–80	12 20		55 75			+10	
Epstein.....	28	M	5 mo.	Rec. alb. +	0	+++	300		50%	6.4	130–80		91	15	less			
	32	F	5 mo.	Rec.	+++	+++	840		74.5%	4.38	130–80		54.8	45	less			
Stepp.....	30	M			0	6 to 8	310				97–	Norm.			2000			
Fanconi.....	2	M	7 mo.	Rec.	+++	10	338			4.9 5.6	100–		32		less			
Karger, Ullmann.	7	F	6½ mo.	Imp. alb. +++	+++ to 0	+++	Milky				95– 110–		31 39					+
Oehlecker.....	15	M		Rec.	+	8 to 12	370 830				Normal				less			+

1058. A large amount of albumin was constantly present. There were some pus cells and many hyaline and granular casts in the sediment. The Wassermann reaction was negative. The basal metabolic rate was + 27 per cent. Blood cholesterol was found to be 372 mg., 383 mg., and 240 mg. on three separate examinations. Urea nitrogen was 13.5 mg. April 17, 16.7 mg. April 25, and 48.5 mg. May 6. The blood chlorides were 460 mg. April 17, 437 mg. one week later, and 435 mg. May 6. The first intravenous phenolsulphonephthalein test gave 14.6 per cent and the second 27 per cent. The serum proteins were approximately 4 gm., of which the globulin fraction was 97 per cent and the albumin fraction only 3 per cent. Repeated blood pressure determinations gave an average of 120/80. However one systolic reading of 140 mm. Hg. was recorded. A few days before death the patient developed fever with abdominal pain, tenderness and rigidity. A diagnosis of peritonitis was made. Death occurred May 7, twenty-eight days after the onset of the edema.

Postmortem: The necropsy reveals generalized anasarca and a diffuse fibrinopurulent peritonitis. Each pleural cavity contains about one liter of cloudy fluid with abundant fibrin on the surfaces. The heart weighs 315 gm., (body weight, 150 lbs.). There is moderate edema of the lungs. The heart and liver show cloudy swelling. The right kidney weighs 250 gm. and the left 260 gm. The capsules are not adherent. The external surfaces are smooth. The cortices are thicker than normal and pale yellow in color.

Microscopic Examination: Sections stained with hematoxylin and eosin show that there is no tubular atrophy and no disease of arteries or arterioles. Most of the convoluted tubules are dilated. There are no epithelial crescents. The glomeruli are enlarged, but they contain very few erythrocytes. The glomerular capillaries are dilated and under low magnification they appear to be empty, but careful study under high magnification shows that most of them are filled with mononuclear cells that have a very pale vacuolated cytoplasm (Fig. 1). Frozen sections stained with Sudan III show these cells filled with fat droplets. There are also some fat droplets in the epithelial cells of the glomeruli. The cells of the convoluted tubules are filled with fat droplets.

The structure of the glomerulus is seen much better in sections stained by Mallory's anilin blue connective tissue stain (Heidenhain, azan-carmine*) (Fig. 2). The cells that fill the capillaries are clearly seen to be of endothelial origin. They have a large amount of vacuolated cytoplasm, the vacuolization being due to fat droplets

* The reader is referred to McGregor's paper for details of the technique and for the histology of the glomeruli in glomerulonephritis.

that have been dissolved out. The majority of the endothelial cells are firmly attached to the basement membrane, but a few are partially or completely free. The basement membrane of the capillaries shows an uneven thickening. The structure differs from typical acute glomerulonephritis in the absence of hyaline fibers in the capillaries but resembles it in that the capillaries are filled with swollen endothelial cells. The capillaries are distended, but the obstruction is sufficient to interfere with renal function. In sections stained with hematoxylin and eosin the glomeruli were considered practically normal, since the extent of the capillary obstruction was not appreciated.

CASE II. *Clinical History:* A–28–5. Female, age 4 yrs. Admitted to the hospital June 21, 1927. The child had been well until about one week before admission when she developed a cold with a nasal discharge. Edema of the ankles appeared June 19, and no urine was passed during the 24 hrs. preceding admission. There was loss of appetite. The urine showed albumin ++++, and hyaline and granular casts. Leucocytes 11,900. Blood pressure 108/64. Temperature 99° F to 100° F. Profuse drainage from both ears. Maxillary sinuses cloudy on roentgenological examination. Blood urea nitrogen 24 mg. On June 25 both sinuses were drained, and the tonsils and adenoids were removed. Pus was found in the left sinus. Edema became very pronounced. July 5, blood pressure 118/80. No fever. General edema. Ascites. Edema varied from time to time and was not relieved by urea, ammonium chloride or thyroid extract. The blood pressure varied from 108/64 to 118/80. June 22, cholesterol 360 mg., blood urea nitrogen 24 mg., nonprotein nitrogen 50 mg.; June 24, urea nitrogen 20 mg., nonprotein nitrogen 62 mg.; June 30, cholesterol 284 mg., urea nitrogen 18 mg., nonprotein nitrogen 37 mg.; July 11, cholesterol 296 mg.; July 13, urea nitrogen 15 mg., nonprotein nitrogen 49 mg. Discharged Aug. 19, 1927, heavy albuminuria, slight edema.

Readmitted, Aug. 23, 1927, because of recurrence of edema. The edema disappeared on two or three occasions but soon reappeared. Albuminuria varied from + to ++++, and there were many pus cells in the urine. Temperature around 99° F. November 8, urea nitrogen 30.4 mg. November 18, total plasma protein 4.41 gm. Albumin:globulin ratio 1.47. December 10, irrigation of sphenoidal sinus, windows made in maxillary sinuses. Total plasma protein, 3.61 gm. Albumin:globulin ratio 1.11. December 13, hemoglobin 74 per cent, erythrocytes 3,890,000, leucocytes 16,050 — 65 per cent polymorphonuclears. December 30, hemoglobin 53 per cent, erythrocytes 3,130,000. Death Jan. 1, 1928, in convulsions and coma.

Postmortem: Emaciation. No edema. No fluid in serous cavities. Early bronchopneumonia. No peritonitis. Kidneys weigh 95 gm. and 105 gm. The capsules strip easily, and the external surfaces are smooth. The cortices are definitely yellowish in color.

Microscopic Examination: The convoluted tubules are moderately distended, and their cells are filled with fat droplets. There is no tubular atrophy, and the blood vessels are normal. There is very little blood in any of the capillaries. The glomeruli are small and bloodless. There are no epithelial crescents.

The modified Mallory stain (Fig. 3) shows many more endothelial nuclei than are visible in the normal glomerulus. In one area a swollen endothelial cell is seen completely filling a capillary. There is no increase of epithelial cells. Very few leucocytes are found in the capillaries. The basement membrane is uniformly thickened and multilayered, and in several areas it shows a very marked thickening. Some of the capillaries are partially or completely obstructed by swollen endothelium or by the thickened basement membrane. The lesion resembles glomerulonephritis to the extent that there is enlargement and increase in number of endothelial cells.

CASE III. *Clinical History:* A–26–625. Female 6 yrs. old. In December 1925 the patient had a mild cold with some fever and malaise which confined her to bed for a few days. She recovered completely and was well until the middle of January 1926, when her eyelids became puffy and her feet and legs became swollen. This edema persisted. About the middle of March 1926 she was very ill for several days with an infection that was called influenza. The temperature was 102° F. Shortly afterward her abdomen became greatly distended. This distention also persisted. She was admitted to the hospital May 18, 1926. There was severe generalized pitting edema and ascites. The urine showed a heavy cloud of albumin with clumps of pus cells and hyaline and granular casts. May 18, urea nitrogen 27 mg., creatinin 1.5 mg., sugar 0.15 per cent. May 19, phenolsulphonephthalein 10 per cent for 2 hrs. (subcutaneous). June 3, urea nitrogen 19 mg., creatinin 1.4 mg., sugar 0.13 per cent. Phenolsulphonephthalein, 15 per cent for 2 hrs. Hemoglobin 68 per cent, red cells 3,680,000. Leucocytes, 16,800 with 56 per cent polymorphonuclears and 42 per cent lymphocytes. Temperature varied from 99.5° F to 101° F. Pulse 100 to 130. June 22, 1000 cc. of clear fluid removed from the abdomen. The patient gradually grew worse and died on July 3, 1926.

Postmortem: The postmortem reveals a generalized purulent peritonitis, a purulent left hydrothorax and early bronchopneumonia. The heart weighs 60 gm. The kidneys weigh together 275 gm. The external surfaces are smooth, and the cortices are of pale color and somewhat thicker than normal.

Microscopic Examination: Marked distention of many of the convoluted tubules. No material was available for fat or other special stains, but the vacuolated cells indicate strongly that abundant fat

was present. No disease of the blood vessels. No tubular atrophy. The glomeruli are small and bloodless. The capillaries are filled with large endothelial cells as in glomerulonephritis.

CASE IV. *Clinical History:* Male 17 yrs. old. Admitted April 8. The chief complaint was a progressive swelling of the legs of about one months duration, and occasional vomiting. No history of an infection of any kind. The physical findings were negative except for marked edema of the legs and moderate ascites. The urine showed albumin ++++. Blood pressure 120/70. The edema subsided somewhat under hospital care, but on April 24 the patient developed the physical signs and symptoms of generalized peritonitis. The edema increased. April 27, blood urea 106 mg. Blood pressure 120/90. April 30, blood urea 144 mg. Leucocytes 21,400. Death May 6.

Postmortem: Generalized purulent peritonitis. Kidneys 415 gm. and 428 gm. The external surfaces are smooth, and the cortices are very cloudy.

Microscopic Examination: No disease of arteries or arterioles. No hyaline glomeruli. No atrophied tubules. A small amount of fat in the convoluted tubules. Moderate dilatation of the convoluted tubules, and marked distention of the capsular spaces. The glomeruli are not enlarged. The capillary lumina appear to be definitely narrowed or collapsed. The anilin blue stain shows an uneven thickening of the basement membrane which has resulted in narrowing of many capillaries and occlusion of a few. There are only a few endothelial cells in the capillaries, and there are no hyaline fibers.

GROUP II. TRANSITION TYPE — NEPHRITIS WITH NEPHROTIC TENDENCY

CASE V. *Clinical History:* A-28-193. Female 18 yrs. old. Patient was a sex delinquent. Chancre was diagnosed Jan. 1, 1926. Intense antisyphilitic treatment was given (neosalvarsan and mercury) from April to July 1927. Sudden onset of edema July 18, 1927. Antisyphilitic treatment was then discontinued. For the next few days she had oliguria and severe pain over the kidneys. Urine contained albumin +++ at this time. She was kept in bed four months on a liquid diet which contained neither salt nor meat. The edema was generalized and did not decrease appreciably under this treatment. The urinary output was small except when she drank large quantities of water. Albumin decreased to +. In October 1927 she was allowed to get up and was given a general diet. However she soon became troubled with nausea and vomiting, and albumin became +++ again. She had frequent attacks of headache throughout her illness. Wassermann reaction positive June 28, 1926, negative October 1, December 6, 1926, and April 1, 1927. Admitted Nov. 22, 1927, to the hospital. Urine at all times showed a large amount of albumin, doubly refractive lipoids,

many hyaline casts and pus cells and occasional red cells. Hemoglobin 99 per cent. Erythrocytes 5,060,000. Leucocytes 11,400 on admission — differential normal. Phenolsulphonephthalein 70 per cent. Blood urea nitrogen varied from 14.9 to 19 mg. The blood pressure was 160/100 for about three weeks during her stay in the hospital. It was normal on admission and shortly before death. Heart normal. Lungs showed signs of congestion. Septic temperature. Death, Feb. 7, 1928.

Postmortem: Massive anasarca. Acute fibrinopurulent peritonitis. Hydrothorax (1200 cc. right, 700 cc. left). Heart 285 gm. Kidneys together 335 gm., surfaces smooth, cortices yellowish.

Microscopic Examination: No atrophy or dilatation of tubules. Extensive hyaline granular degeneration of many convoluted tubules. Abundant fat in all the convoluted tubules. No disease of arteries or arterioles. The glomeruli are of normal size and contain a relatively small amount of blood so that there appears to be an increase of cells at the expense of the lumina of the capillaries. The anilin blue stain (Fig. 4) shows that most of the nuclei belong to enlarged epithelial cells which compress the capillaries, but there is a definite increase in the number of endothelial nuclei. The basement membrane is definitely thicker than normal. The glomerular lesion consists chiefly in enlargement of the epithelial cells and thickening of the basement membrane with resulting narrowing of the capillaries.

The only clinical distinction from pure lipoid nephrosis is the presence of hypertension.

CASE VI. *Clinical History:* A–28–183. Female, 34 yrs. old. Well until June 1, 1927, when she first noticed unusual fatigue and drowsiness. On June 15 she noticed swelling of the ankles, and the next day her legs were swollen also. In the latter part of July she developed an acute respiratory infection and was very ill with general anasarca, vomiting and suppression of urine. She was told at this time that there was albumin and pus in the urine. Hospitalized for two months. Repeated abdominal paracentesis. Admitted to our service Jan. 4, 1928, complaining of fatigue, drowsiness, anasarca, vomiting and headache. Blood pressure 182/130. Distended abdomen (fluid). Albuminuria with casts. Hemoglobin 48 per cent. Erythrocytes 2,690,000. Leucocytes 9,300. Eyegrounds negative at first but a retinal hemorrhage was observed three weeks later. January 5, urea nitrogen 24.2 mg., creatinin 2.2 mg.; January 9, urea nitrogen 26 mg., creatinin 2.6 mg.; January 18, urea nitrogen 31.2 mg., creatinin 3 mg.; January 30, urea nitrogen 47 mg., creatinin 7.2 mg. Blood pressure January 9, 200/140; January 12, 204/140; January 18, 208/140. The edema gradually decreased under diuretics and low fluid intake, but death occurred on Feb. 6, 1928.

Postmortem: Edema of face, none elsewhere. Ascites. Heart 300 gm. Early bronchopneumonia. Acute rheumatic mitral endocarditis. Left kidney absent. Right kidney 300 gm., external surface smooth, cortex pale.

Microscopic Examination: Moderate diffuse tubular atrophy (Fig. 5). No hyaline glomeruli. No disease of arteries or arterioles. Moderate amount of fat in the convoluted tubules. Dilatation of some of the tubules. The glomeruli are not enlarged, but there is evidently an increased number of nuclei. The anilin blue stain (Fig. 6) shows a great many capillaries partly occluded by large endothelial cells and polymorphonuclear leucocytes. The most striking change is a marked, irregular thickening of the basement membrane, which causes narrowing and sometimes complete occlusion of the capillaries. There is no change in the epithelial cells of the glomerulus. The extensive narrowing and occlusion of the glomerular capillaries are responsible for the tubular atrophy. The obstruction in the glomerular circulation is fairly uniform but none of the glomeruli are completely occluded. The result is a moderate diffuse tubular atrophy with no hyaline glomeruli and no completely atrophied tubules. One enlarged glomerulus was found which showed complete occlusion of all the capillaries by endothelial cells and hyaline fibers, such as is found in typical glomerulonephritis.

CASE VII. *Clinical History:* A–17–145. Male 54 yrs. On the night of Aug. 19, 1916, after working in a cold damp place he suddenly developed edema of the feet. He continued to work, but the swelling grew worse. On September 7 he first noticed edema of both lower eyelids. Admission, Sept. 14, 1916. The urine showed albumin ++, hyaline and granular casts. Hemoglobin 75 per cent, erythrocytes 5,800,000, leucocytes 8000. Blood pressure 155/80. Wassermann reaction negative. Phenolsulphonephthalein, September 15, 72 per cent; September 29, 52 per cent. September 15, urea nitrogen 13 mg., creatinin 4 mg., sugar 0.16 per cent. Discharged Nov. 25, 1916. Readmitted May 17, 1917. On the second admission he complained of marked swelling of the feet and back, severe dyspnea, and gastric distress. He stated that he had felt drowsy and tired most of the time since he left the hospital. Urine: albumin ++, granular and hyaline casts. Hemoglobin 75 per cent, erythrocytes 4,000,000, leucocytes 8000. Blood pressure 190/95; 175/95. July 16, blood pressure 125/95. Wassermann reaction negative. Phenolsulphonephthalein May 19, 35 per cent; July 7, 20 per cent. Urea nitrogen, June 11, 23.7 mg., creatinin 3.2 mg., sugar 0.11 per cent. Death July 21, 1917.

Postmortem: Severe general anasarca, marked ascites and hydrothorax. Edema of lungs, purulent bronchitis and atelectasis. Heart

320 gm. Kidneys together weigh 450 gm., smooth external surfaces, thickened yellowish cortices.

Microscopic Examination: There are a few hyaline glomeruli with atrophic tubules. Many of the convoluted tubules are dilated, and all of them contain fat droplets. There is no disease of arteries or arterioles. The glomeruli are of normal size and contain only a few erythrocytes. Many of them show adhesions to the capsule (Fig. 7). The anilin blue stain shows a general narrowing of the capillaries due chiefly to enlargement of the epithelial cells and thickening of the basement membrane. There are some swollen endothelial cells in the capillaries. The structure is about the same as is shown in Fig. 4. At the site of the adhesions a few capillaries contain hyaline fibers and swollen endothelium as in typical glomerulonephritis.

Group III. Non-Contracted Kidneys of Uncertain Type

These probably belong with the lipoid nephroses, but the available clinical data are insufficient for a definite diagnosis.

Case VIII. *Clinical History:* A–27–511. Female, 43 yrs. old. Present illness began 1 yr. ago with pain in the right upper quadrant and vomiting. Since that time she has had repeated attacks of dyspnea and edema of the ankles. No other past history available. Admitted to the hospital May 6, 1927, 12 hrs. before death, in coma. Severe dyspnea. Slight edema of the ankles. No cardiac murmurs. Blood pressure 110/70. Urine: trace of albumin, no sugar, occasional hyaline casts.

Postmortem: Heart weighs 538 gm., left ventricular hypertrophy, no valvular lesions. No fluid in the serous cavities. Marked edema and congestion of lungs. Liver weighs 2730 gm. and shows moderate fatty metamorphosis. The left kidney weighs 304 gm. The capsule strips easily. The external surface is smooth. The cortex is of grayish yellow color. (No note was made on the right kidney.)

Microscopic Examination: No tubular atrophy. No hyaline glomeruli. Small arteries show a marked increase of elastic tissue. No hyalin in the arterioles. Moderate dilatation of all convoluted tubules A small amount of fat in the convoluted tubules. The glomeruli are large and anemic and show a great decrease in the capillary bed (Fig. 8). The anilin blue stain (Fig. 9) shows a marked irregular thickening of the basement membrane which has resulted

in narrowing of all the capillaries and complete closure of a few. There is a notable increase in the number of endothelial nuclei. Epithelial cells are inconspicuous.

The marked left ventricular hypertrophy indicates that hypertension was present for a long time. The low blood pressure found shortly before death does not exclude a previous hypertension. The possibility that this is a primary hypertension must be considered, but none of the known cases of this disease shows such striking glomerular lesions in the absence of arteriolar disease. The glomerular lesions have some resemblance to advanced chronic glomerulonephritis.

CASE IX. *Clinical History:* A–26–827. Female 62 yrs. old. Admitted Sept. 11, 1926, complaining of generalized edema and dyspnea. Fifteen years ago she was confined to bed for one year with generalized edema. No further information about this attack could be obtained. From that time on her health was fair except for occasional attacks of edema of the ankles and some "stomach trouble." About Aug. 1, 1926, the edema became worse, and she noticed swelling of the abdomen, dyspnea, orthopnea and scanty urine. Her tongue was sore, and she noticed numbness of the hands occasionally. On admission her temperature was 97.7° F; pulse 100; respirations 30; blood pressure 134/82. Dyspnea, edema and weakness were pronounced. The skin and mucosae were pale. Soft systolic murmur at the apex. Ascites. Knee jerks absent. Babinski negative. Hemoglobin 40 per cent. Erythrocytes 1,310,000. The smear showed anisocytosis, poikilocytosis, macrocytes, nucleated reds and polychromatophilia. Leucocytes 2500. Neutrophils 59 per cent, lymphocytes 21 per cent, monocytes 20 per cent. Urine: albumin +++, hyaline and granular casts and a few erythrocytes. Blood urea nitrogen 25.2 mg., creatinin 1.36 mg. Death Sept. 12, 1926.

Postmortem: Marked general anasarca; ascites (7000 cc.); bilateral hydrothorax (150 cc. each). Heart 330 gm. Spleen 37 gm. Red bone marrow in the shafts of the femurs. Kidneys together weigh 260 gm., external surfaces smooth, cortices of normal thickness.

Microscopic Examination: Sclerosis of the larger branches of the renal arteries, but no involvement of small arteries or arterioles. No tubular atrophy. Slight dilatation of the convoluted tubules. The cells of the convoluted tubules contain a small amount of fat and a large amount of blood pigment. All the glomeruli have a hyaline appearance (Fig. 10). The lumina of the capillaries are largely obliterated. The anilin blue stain (Fig. 11) shows that the extreme narrowing of the capillaries is due to a massive thickening of the base-

TABLE

Summary of Clinical Data in

	Case number	Sex	Age in yrs.	Duration	Edema	Albuminuria	Cholesterol mg. per 100 cc.	Serum albumin	Serum globulin	Total protein	Blood pressure	Blood urea mg. per 100 cc.	Urea nitrogen mg. per 100 cc.	N.P. nitrogen mg. per 100 cc.	Phenolsulphonephthalein
Pure lipoid nephrosis	I	M	28	4 wk.	+++	++++	372 383 240	0.12	3.88	4	120–80 140–		13.5 16.7 48.5		14.6 27
	II	F	4	5½ mo.	o to +++	++++	360 284	2.63 1.90	1.78 1.71	4.41 3.61	108–64 118–80		24.0 20.0 18.0 15.0 30.4	50 62 37 49	
	III	F	6	6 mo.	+ to +++	++							27.0 19.0		10 15
	IV	M	17	7 wk.	+++	++++					120–70	106 144			
Mixed type	V	F	18	7 mo.	+ to +++	+ to ++++					160–100		14.9 19.0		70
	VI	F	34	7 mo.	+++ to +	+++					182–130 208–140		24.2 31.2 47.0		
	VII	M	54	1 yr.	+ to +++	++					155–80 175–95 190–95		13.0 23.7		72 52 35 20
Uncertain types	VIII	F	43	1 yr.	+	+					110–70				
	IX	F	62	15 yr. (6 wk.)	o to +++	+++					134–82		25.2		
	X	F	52	2 yr.	++										

III

Ten Cases Studied at Postmortem

Oliguria	Wassermann	Basal metabolic rate	Peritonitis	Weight of kidneys in gm.	Possible etiological factors	Fat in tubules	Glomerular lesion
+	−	+27	+	510	Common cold	+++	Capillaries partly obstructed by enlarged fatty endothelial cells. (Figs. 1 and 2)
+			−	200	Common cold, nasal discharge, otitis media, sinusitis.	+++	Swollen endothelium, thickened basement membrane. (Fig. 3)
			+	275	Common cold (?)	+++	Swollen endothelium.
			+	843		+	Uneven thickening of basement membrane, slight endothelial swelling.
+	+		+	335	Antisyphilitic treatment.	+++	Thickened basement membrane, enlarged epithelial cells. (Fig. 4)
			−	300		++	Swollen endothelial cells, thickened basement membrane, rarely glomerulonephritis. (Figs. 5 and 6)
	−		−		Exposure to cold and dampness.	++	Thickened basement membrane, enlarged epithelial cells, focal glomerulonephritis. (Fig. 7)
			−	304(?)		+	Marked thickening of basement membrane, increase of endothelium. (Figs. 8 and 9)
			−	260		+	Extreme thickening of basement membrane, increase of endothelium. (Figs. 10 and 11)
			+	350		++++	Very large fatty and hyaline epithelial cells, glomerulonephritis. (Fig. 12)

ment membrane. The nuclei all seem to belong to endothelial cells. There is a close resemblance to Case VII (Fig. 8), but the thickening of the membrane is more pronounced and the endothelial cells are less conspicuous.

It is not clear whether death was due to anemia or to renal insufficiency.

CASE X. *Clinical History:* A–27–305. Female 52 yrs. old. History of edema of the legs and ankles for the past two years. She first noticed distention of the abdomen on March 15, 1927. At the same time she had cramp-like abdominal pains and vomited throughout the day. On March 16, she continued to have acute abdominal pain but did not vomit. Admitted March 18. Rapid labored respiration. Crackling râles at the bases of the lungs. Abdomen distended and tender but not rigid. Edema of legs and feet. Temperature 101.8° F. Leucocytes 21,000 — 79 per cent polymorphonuclears. Hemoglobin 80 per cent. Erythrocytes 4,260,000. Death March 18.

Postmortem: Diffuse purulent peritonitis. Heart 315 gm. Edema of lungs. Kidneys together weigh about 350 gm., surfaces smooth, cortices yellowish.

Microscopic Examination: Large numbers of fat droplets in the convoluted tubules, glomeruli and interstitial connective tissue cells. The glomerular fat is very prominent and is situated chiefly in the epithelial cells within the glomerulus and on its surface. About 10 per cent of the glomeruli are hyaline, and these are associated with completely atrophied tubules. The other tubules show no atrophy or dilatation. There is no arterial or arteriolar disease. The glomeruli are large and have an unusual appearance (Fig. 12). The lumina of the capillaries are either greatly narrowed or completely occluded. Large hyaline granules are seen which are chiefly situated within the epithelial cells. The anilin blue stain shows very large epithelial cells filled with hyaline masses. The occluded capillaries are filled with endothelial cells and a network of hyaline fibers such as are found in typical glomerulonephritis. The enlargement of the glomeruli and the narrowing of the capillary lumina are due to two processes: (*a*) marked enlargement of the epithelial cells with fatty and hyaline degeneration, and (*b*) enlargement of endothelial cells and formation of hyaline fibers within the lumina of the capillaries. The disease may be interpreted as a modified form of typical glomerulonephritis.

A summary of the clinical and anatomical data in the ten cases just described is given in Table III.

Discussion of the Various Phenomena of Lipoid Nephrosis

1. *Albuminuria* is a constant finding. Edemas without protein in the urine are not nephroses. In the early stages, in the remissions and in the stage of recovery there may be only a trace, but usually the amount of protein is very large. Amounts as high as 6 per cent have been reported. Epstein estimated that as much as 50 gm. of protein may be excreted daily, and since the total protein in the blood is only about 210 gm., he considers the loss of blood protein one of the most important phenomena of the disease.

Epstein believes that the loss of blood protein in the urine is the cause of the edema in that it results in a decrease of the osmotic pressure of the blood, but there are so many instances of low plasma protein without edema, and *vice versa*, that a causal relationship between albuminuria and edema is uncertain.

2. *Oliguria* is usually noted when the disease is at its peak, but normal or increased output occurs when the edema is lessening. A twenty-four hour excretion of 200 to 800 cc. of urine is frequently seen. A period of anuria was observed in only one case (Kaufmann and Mason).

3. *Lipoiduria* is considered by Munk as pathognomonic of lipoid nephrosis. In his first publication he seems to have considered albuminuria, edema and lipoiduria as sufficient to establish the diagnosis. Doubly refractive bodies are readily demonstrated by attaching a polariscope to an ordinary microscope.

Reference to the tables will show that lipoids were found in the urine in 16 of 19 cases in which they were looked for. Lipoiduria is therefore a rather constant finding in lipoid nephrosis. But Genck has shown that not all doubly refractive bodies in the urine are of fatty nature. Spherocrystals of various kinds are doubly refractive. Lipoiduria has been found in glomerulonephritis (Laweynowicz, 2 of 12 acute cases; Genck, 1 of 6 acute cases; Finger and Kollert, 32 of 76 acute or subacute cases, and 5 of 21 chronic cases; Kollert and Finger, 101 of 289 war nephritics [glomerulonephritis]). It has also been found in diabetes, amyloid disease and pyelonephritis. Tietz found lipoiduria in renal diseases of various types. It is obvious therefore that lipoiduria is not peculiar to lipoid nephrosis.

4. *Lipemia:* An increase of the blood lipoids is found constantly in lipoid nephrosis and usually in glomerulonephritis with edema. The milky serum of nephritics was known to Richard Bright. Erben, in 1904, called attention to the high fat and lecithin content of the blood in nephritics. Chauffard, Laroche and Grigaut in 1911 found hypercholesterolemia in cases of renal edema with albuminuria and noted also that there was no increase of cholesterol in nephritics with nitrogen retention. Epstein has published a series of papers dealing with lipoid nephrosis and glomerulonephritis with a nephrotic tendency. He found hypercholesterolemia a characteristic feature.

Values from 300 to 600 mg. per 100 cc. of blood are commonly reported. Rarely much higher values are recorded (1000 mg. Hahn and Wolff; 1350 mg. Epstein and Lande). Often the serum is of milky appearance, but a high lipoid content may be found when the serum does not have this cloudy appearance (Hahn and Wolff).

The increase of blood lipoid is largely represented by cholesterol, but there is also a marked increase of lecithin and fatty acids (Knaur, Daniels).

Strauss and Schubardt found cholesterol values of over 200 mg. in 6 of 17 cases of acute glomerulonephritis, and in 11 of 44 chronic cases.

Lipemia and renal edema are closely associated. Bennett doubts the occurrence of renal edema without hypercholesterolemia. Maxwell in an extensive study of nephritis found hypercholesterolemia in both acute and chronic forms whenever edema was present. He found only one exception to the rule that renal edema never occurs without hypercholesterolemia.

When uremia develops in chronic glomerulonephritis, the cholesterol sinks to a normal level (Chauffard, Stepp, Maxwell).

Amyloid disease of the kidneys usually shows lipemia. The blood lipoids are usually slightly increased during pregnancy, and in eclampsia there is often a definite increase above the normal level of pregnancy (Hinselmann). An increase has also been noted in sublimate poisoning (Wichert and Russjajewa-Oparina).

Lipemia is not restricted to renal disease. A moderate increase of blood lipoids is often found in diabetes, and sometimes a very marked lipemia is observed. In 25 diabetics Bing and Heckscher found normal lipoid values in 11, moderate increases in 13, and a severe lipemia in one. Strauss and Schubardt found lipoid values in diabetics regularly about 250 mg.

There is often some increase of lipoids in obstructive jaundice (Stepp, Strauss and Schubardt).

Increase of the blood lipoids is a characteristic feature of lipoid nephrosis, but this finding alone does not differentiate this disease from glomerulonephritis with edema, from amyloid nephrosis, or even from certain extrarenal diseases.

Edema may occur without lipemia and *vice versa*, but the close association of edema and lipemia in renal disease suggests a common etiology. Little is known of the underlying causes of lipemia. The fats are believed to come from the ingested food since a pronounced lipemia may be produced in rabbits by intensive cholesterol feeding (Thölldte). It has been suggested that the lipemia in renal disease is due to an inability to utilize fat as it presumably is in diabetes, but Hiller, Linder, Lundsgaard and Van Slyke find that nephritics with constant lipemia burn fat as efficiently as normals.

5. *Edema:* The diagnosis of lipoid nephrosis should not be made if edema has not been present at any time. Apparently only two authors have made this diagnosis in the complete absence of edema, *viz.*, Stepp and Epstein. Epstein's case is obviously chronic glomerulonephritis, and the data in Stepp's case are insufficient for accurate diagnosis. However, in typical cases edema may be absent in the early stages and in the remissions, and it may disappear spontaneously or as a result of treatment.

Edema is commonly severe at the peak of the attack and is often the chief cause of the patient's discomfort. Therapeutic procedures should therefore be directed toward the relief of this symptom when it is pronounced. In general the most successful therapy appears to be a diet with restricted salt and fluid content, and the liberal use of mild diuretics. Epstein strongly recommends a high protein diet on the theory that it will replace the blood proteins lost in the urine. He believes that the edema is due to the loss of plasma proteins in the urine which lowers the osmotic pressure of the blood and results in an excessive accumulation of fluid in the tissue spaces. Edema fluids in nephrosis are poor in protein.

6. *Retention of Sodium Chloride:* There is a retention of sodium chloride in nephrotic edema. It is apparently held in the tissues since it is not increased in the blood. There is no satisfactory evidence that the ability of the kidney to excrete water or salts is seriously impaired. It is possible that edema is due to an injury of the

tissue cells throughout the body which causes them to retain water and crystalloids in the tissue spaces.

7. *Decrease of Plasma Proteins:* Richard Bright knew that the plasma proteins were decreased in nephritis. Erben, in 1904, records a decrease of albumin and globulin and an increase of fibrin in "chronic parenchymatous and subchronic nephritis." In 1905 he observed a shift of the albumin:globulin ratio in favor of globulin so that globulin sometimes exceeded albumin.

Epstein has made extensive studies on the plasma proteins and found them characteristically decreased with reversal of the albumin:globulin ratio in pure lipoid nephrosis as well as the mixed type. In the 10 cases listed in Tables I and II, in which the proteins were studied, they were found decreased in 8. The total protein was greatly decreased in the 2 cases of my series that were studied (Table III), but the albumin:globulin ratio was not reversed in one of them.

The protein lost is almost entirely albumin. Epstein gives an example of a nephrosis in which albumin was 0.466 mg., and globulin 3.462 mg. In one of my cases (Table III) the total protein was 4 mg. of which albumin was 3 per cent and globulin 97 per cent. The normal albumin:globulin ratio is 1.4 to 1.8. Linder, Lundsgaard and Van Slyke found reduction of the plasma proteins with a low albumin:globulin ratio in chronic nephrosis and chronic nephritis with edema but not in nephrosclerosis (hypertension kidney). Fahr and Swanson found a definite reduction of the total plasma protein largely due to loss of serum albumin in 7 of 10 cases of acute and subacute glomerulonephritis. In 5 of 9 cases of chronic glomerulonephritis the total protein was below 6 mg. Decrease of plasma protein evidently does not differentiate lipoid nephrosis from glomerulonephritis.

Epstein believes that edema is due to loss of albumin in the urine and consequent decreased osmotic pressure of the plasma. Low plasma protein and edema are both characteristic of lipoid nephrosis, but they are often not present simultaneously. A low protein level may persist long after edema has disappeared, and intense edema may develop before the blood proteins show any change. It seems more probable that both conditions are due to some underlying cause than that edema is caused by the low protein level.

A decrease of plasma proteins with reversal of the albumin:globulin

ratio has been found in lobar pneumonia in the complete absence of edema and with only a trace of protein in the urine (Geill, Kumpf). Evidently a decrease of protein may occur without a loss in the urine. It has not been proved that the regeneration of plasma proteins is hastened by a high protein diet.

8. *Hematuria:* Macroscopic blood is never found in lipoid nephrosis, but some observers have noticed a few erythrocytes in microscopic preparations. Glomerulonephritis commonly does not show hematuria except in the acute stages and during exacerbations.

9. *Chloride Retention:* When edema is prominent there is usually a decreased elimination of chlorides in the urine. Since there is no increase in the blood it is inferred that the chlorides are retained in the tissues. There is a similar chloride retention in glomerulonephritis with marked edema.

10. *Basal Metabolism:* The basal metabolic rate was low in 13 of the 15 cases in which it was studied (Tables I and II), but this is probably of little significance since the authors do not make corrections for the weight of the edema fluids. G. Fahr* has shown that the basal metabolic rate is usually normal if calculations are based upon the normal weight of the individual.

Epstein believes that certain nephroses are related to myxedema and that they respond favorably to thyroid medication.

11. *Renal Function:* On this point a distinction between lipoid nephrosis and chronic glomerulonephritis is usually made. Whenever a definite nitrogen retention is demonstrable it is assumed that the case is not pure nephrosis but a mixed type (nephritis with nephrotic *Einschlag*). But the various authors do not agree as to the nitrogen level that is admissible to the nephrotic group. It is to be remembered that ordinary mild cases of chronic glomerulonephritis often show no nitrogen retention, and it is uncommon to find urea nitrogen much above the normal except in advanced stages of the disease.

Bannick and Keith in using a level of 40 mg. of blood urea to separate nephrosis from nephritis may have included some cases of glomerulonephritis with the nephroses. In three instances (Table I), in which the postmortem report was pure lipoid nephrosis, there was a definite nitrogen retention. In one of my cases (Case I, Table III)

* Personal communication.

which corresponds to pure lipoid nephrosis in every other respect, the last blood urea nitrogen was 48.5 mg.

It is clear that some cases that are anatomically pure lipoid nephrosis have a moderate nitrogen retention and that many mild cases of chronic glomerulonephritis do not. No instance of pure lipoid nephrosis with uremia has been reported. It may be concluded that renal edema with uremia is not pure lipoid nephrosis, and that with moderately increased or normal blood nitrogen it may or may not belong in this group.

Phenolsulphonephthalein: In the cases reported (Tables I and II) the phthalein excretion is moderately diminished in most instances. The lowest observation was 15 per cent. When phthalein is injected intramuscularly, edema interferes with its absorption and tends to give too low a reading. This may have been the cause of the low reading in Case III (Table III), but in Case I (Table III) the phthalein was injected intravenously. The results with phthalein indicate a moderate impairment of renal function. Chronic glomerulonephritis also shows only a moderate decrease of phthalein elimination before the final stage begins.

12. *Blood Pressure:* It is commonly assumed that when the systolic blood pressure is above 140 mm. Hg. in a case which otherwise has the characteristics of lipoid nephrosis, it is a mixed type, *i. e.*, nephritis with nephrotic *Einschlag*. Volhard states that hypertension is the only symptom by which nephritis with nephrotic *Einschlag* may be distinguished clinically from pure nephrosis. Several authors have reported clinical cases with high blood pressure as lipoid nephrosis, but these have been omitted from Table II. Apparently no extensive studies have been made to determine whether there is an anatomical basis for the exclusion of a case from the "pure lipoid nephrosis" group because of hypertension.

One often sees acute and mild chronic glomerulonephritis with a systolic blood pressure below 140 mm. Hg. There is no justification for classifying all of these as lipoid nephrosis.

13. *Causes of Death in Lipoid Nephrosis:* In the postmortem reports (Table I) it will be noted that eleven of the twenty-four patients died of peritonitis. In the cases in which bacteriological studies were made the invading organisms were found to be pneumococci. Volhard states that all his fatal cases died of pneumococcic peritonitis. Three of my four cases of the pure type and

one of three of the mixed type died of peritonitis (Table III). The tendency to develop peritonitis seems to be characteristic of pure lipoid nephrosis and the mixed type. Of fifty-three examples of typical subacute and chronic glomerulonephritis that I have studied, none died of peritonitis.

Over half of the fatal cases reported died of infections other than peritonitis, such as pneumonia and septicemia. None died of uremia.

14. *Frequency of Lipoid Nephrosis:* Volhard states that he has seen about a dozen cases. Epstein observed 15 cases of "chronic nephrosis" among 193 cases of renal disease, but "chronic nephrosis" includes amyloid kidney, pure lipoid nephrosis and nephrosis with nephrotic *Einschlag*. McElroy diagnosed lipoid nephrosis nineteen times in 600 cases of Bright's disease. Schlayer saw only 6 cases in 300 cases of nephritis and was uncertain of some of these. Bannick and Keith report a surprisingly high incidence — 25 cases of "chronic nephrosis" and 25 cases of "chronic nephrosis of mixed type" in about 90 cases of nephritis. The high incidence noted by these authors is evidently due to their having included as "chronic nephrosis" many cases which others would regard as ordinary chronic glomerulonephritis.

I have made postmortem studies on 53 cases of subacute and chronic glomerulonephritis, 3 that may be called glomerulonephritis with nephrotic *Einschlag*, 4 of pure lipoid nephrosis and 3 of uncertain classification.

15. *Duration and Prognosis:* The duration of the 24 fatal cases (Table I) was as follows: 1 mo., 3; 1–3 mo., 5; 3–6 mo., 3; 6 mo.–1 yr., 6; 1–3 yr., 7. Six cases are recorded (Table II) as having recovered completely, but the great majority still had albuminuria at the time of the report. Munk thinks that most cases of lipoid nephrosis end in healing but albuminuria may last for years or decades. He mentioned one patient who had albuminuria for ten years but was entirely well fifteen years after the onset of her illness. Steinitz thinks that the prognosis is good as to life but poor as to recovery from albuminuria. Volhard mentioned 6 fatal cases among more than a dozen that he had seen.

The duration of my 4 cases of pure lipoid nephrosis was four weeks, seven weeks, five and a half months, and six months, respectively. The 3 cases of mixed type lasted seven months, seven months, one

year respectively (Table III). Dr. G. Fahr has furnished me the record of a patient he has had under observation for four years. He was confined to the hospital about one year, with all the typical features of pure lipoid nephrosis. He left the hospital practically recovered and was entirely free of all signs of the disease three years later.

Examples may be found in the older literature of "chronic parenchymatous nephritis" that recovered. Some of these are no doubt instances of what is now called lipoid nephrosis.

16. *Pathological Anatomy:*

(*a*) *Pure Lipoid Nephrosis:* The kidneys are always enlarged and pale. In one of my cases the combined weight of the two kidneys was 843 gm. The external surfaces are smooth. The color of the cortex varies from pale to yellow. There are always some lipoid droplets in the convoluted tubules and they are often in the glomerular epithelium also, but the amount of fat is not always excessive. There may be only a small amount of fat insufficient to give the cortex a yellow color. The convoluted tubules and capsular spaces are usually dilated. An outstanding feature is the absence of tubular atrophy. Often many of the convoluted tubules are filled with coarse hyaline granules, and these granules may also be found in the epithelial cells of the glomerular tuft both on the surface and between the loops.

The glomeruli are described as normal by nearly all the authors who have reported cases of pure lipoid nephrosis. In fact, normal glomeruli are generally considered necessary to establish the diagnosis of the pure type. However, some observers have realized that they are not entirely normal. Stolz finds them almost normal but containing polymorphonuclear leucocytes. Major and Helwig noted that some glomeruli showed swollen endothelial cells and others contained lipoid droplets. Munk finds no changes at first except some increase of leucocytes. He states, however, that later a definite glomerulitis may develop that leads to a contracted kidney. Volhard finds that the glomerular capillaries are not occluded, but the cells may contain hyaline, granular or lipoid droplets. Fahr noted minor lesions in individual glomeruli in some of his cases. He mentions very slight proliferation, hyaline clumps, collapsed capillaries and fusion between the glomerulus and its capsule.

In sections stained with hematoxylin and eosin the glomeruli appear to be normal except for an increased number of leucocytes in

some of the capillaries, but in sections stained by the anilin blue method definite abnormalities can be made out. In Case I the capillaries were filled with swollen endothelial cells heavily laden with lipoid droplets (Fig. 2). This corresponds with clinical glomerulonephritis except for the absence of hyaline fibers in the capillaries. Case II showed some increase of endothelium and a patchy thickening of the basement membrane (Fig. 3). No special stain could be made on Case III. Case IV showed a fairly diffuse thickening of the basement membrane with narrowing of the capillaries and some increase of endothelium.

The lesions are not exactly the same in any two instances, but they all show enlargement and increase in number of the endothelial cells in some degree as well as thickening of the basement membrane.

In clinical glomerulonephritis the characteristic lesion is a plugging of the capillaries with swollen endothelial cells among which are many hyaline fibers (McGregor). There is therefore a fundamental resemblance to glomerulonephritis, but the endothelial swelling is not so pronounced, there are no hyaline fibers and the capillary lumina are only partially obstructed.

(*b*) *Nephritis with Nephrotic "Einschlag," (Nephritis with Nephrotic Tendency — Mixed Type)*: Clinically this type is defined as a disease with all the features of pure lipoid nephrosis except for the presence of hypertension or definite renal insufficiency. I have not found any descriptions of the pathological changes in the kidneys. Cases V, VI, and VII belong to this group. They differ from pure lipoid nephrosis clinically only in the presence of hypertension. Macroscopically the kidneys cannot be distinguished from those of pure lipoid nephrosis.

On microscopic examination lipoid droplets are found in the tubules. The glomeruli show transitions to typical glomerulonephritis in that entire glomeruli (1 case) or parts of glomeruli (1 case) may show the characteristic lesion of glomerulonephritis. Increase in the size and number of the endothelial cells was conspicuous in 2 cases, and thickening of the basement membrane was noted in all 3. Enlargement of the glomerular epithelial cells was the most conspicuous change in one case. The mixed type, nephritis with nephrotic *Einschlag*, therefore resembles both pure lipoid nephrosis and typical glomerulonephritis in that endothelial increase is one of its histological features. The histological structure is not fundamentally

different from that of pure lipoid nephrosis, but there is more obstruction in the capillary bed.

On histological grounds one cannot make a sharp distinction between glomerulonephritis and either the mixed or pure type of lipoid nephrosis. There is a fundamental resemblance in that all three types show endothelial swelling and increase. The differences seem to be in degree rather than in kind.

(*c*) *Renal Lesions of Uncertain Type:* I have studied 3 cases in which the clinical data are inadequate to establish a clinical diagnosis. The kidneys are large and fatty, and there is no tubular atrophy. In the older terminology they would be called chronic parenchymatous nephritis. The glomerular lesions are very prominent. In Case VIII (Figs. 8 and 9) the glomeruli are large, and there is an increase of endothelial nuclei and a marked thickening of the basement membrane with narrowing of the capillaries. In Case IX (Figs. 10 and 11) the thickening of the basement membrane is so extreme that the capillaries are almost obliterated. The clinical picture in this case was that of lipoid nephrosis, *viz.*, severe albuminuria, edema, normal blood pressure and no nitrogen retention, associated with pernicious anemia. However, there was no determination of blood lipoids, and the kidneys had only a small amount of fat.

In Case X (Fig. 12) the epithelial cells are greatly enlarged and they show an extreme hyaline and fatty degeneration. The capillaries contain large endothelial cells and hyaline fibers as in clinical glomerulonephritis. This case is closely related histologically to glomerulonephritis. The enormous accumulation of fat in the kidneys, the edema and the peritonitis are almost sufficient to put it in the clinical group of lipoid nephrosis.

These 3 cases raise the question whether all cases with albuminuria, edema, normal blood pressure and large fatty kidneys should be regarded as lipoid nephrosis regardless of the type of glomerular lesion present.

17. *Etiology:* Munk laid great emphasis upon lues as a causative factor but did not think that all cases were due to lues. Lues is considered an important etiological factor by Volhard, Fahr, Felber, and Karger and Ullmann. However, it is shown in Tables I, II and III that among twenty-one patients examined for lues only three were found to be positive. Syphilis cannot therefore be considered of any particular importance in the etiology of lipoid nephrosis.

Several authors hold the view that lipoid nephrosis is a general metabolic disturbance in which the kidney is affected but that it is not a primary renal disease. Kollert believes that an infection or an intoxication causes a prolonged injury of numerous body cells which expresses itself as a metabolic disturbance. The renal injury is a part of the picture. He offers the same explanation for nephritis with nephrotic *Einschlag*. Knaur does not consider lipoid nephrosis a renal disease. He believes that the primary disturbance is hypotension with resulting poor filtration of water. Schlayer attributes the disease to universal capillary injury. Epstein and Löwenthal regard it as a constitutional disease with secondary renal changes, but not restricted to the kidney. The features which suggest a constitutional disease are the general disturbances such as edema, hypercholesterolemia, alterations in the plasma proteins, etc.

Volhard and Fahr seem to consider lipoid nephrosis as essentially a disease of the renal tubules.

Löhlein, in 1918, expressed the opinion that nephrosis and nephritis with nephrotic *Einschlag* begin as glomerulonephritis and may end with uremia and retinitis. Elwyn has observed several cases of lipoid nephrosis which began as acute diffuse glomerulonephritis.

Clausen stresses the importance of sinus infections. Two of my cases and possibly a third began with a common cold, and another followed exposure to cold and dampness (Table III). This is not unlike the history of acute glomerulonephritis and suggests that infection may be the causative factor.

18. *Is Lipoid Nephrosis Primarily a Renal Disease?* The writers who contend that the disease is a general metabolic disorder with a secondary renal disturbance have not properly appreciated the degree of the renal injury. The glomeruli are regarded as normal, and the only renal lesion is considered to be lipoid infiltration of the tubules. The disturbance of lipoid metabolism and the changes in the blood proteins are regarded as strong arguments in favor of a general disorder.

However, the same disturbances in the blood fats and proteins occur in glomerulonephritis, which is certainly a primary disease of the kidneys. Lipoid nephrosis shows heavy albuminuria and minor disturbances of renal function which must be interpreted as indicating renal damage. There are definite and sometimes pronounced structural changes in the glomeruli, and there are transitions to glo-

merulonephritis both clinically and anatomically. Lipoid nephrosis appears to be a renal lesion in which there is partial but not complete obstruction of the glomerular circulation. Uremia does not develop, but in some unknown way there develops an increased susceptibility to infection, especially to peritonitis.

The Relation of Lipoid Nephrosis to Glomerulonephritis

Clinical Relation: It is obvious from the clinical literature that a diagnosis of lipoid nephrosis is made much more frequently by some physicians than by others. The usual requirements for the diagnosis are albuminuria and edema without definite hypertension or nitrogen retention. When this standard is applied to acute renal disease it undoubtedly includes a great many cases of acute glomerulonephritis, and when applied to chronic renal disease it includes some mild cases of chronic glomerulonephritis. When defined in this way lipoid nephrosis is a fairly common ailment. If, however, we require for the clinical diagnosis severe albuminuria, marked edema, normal or low blood pressure, no nitrogen retention, hypercholesterolemia and low plasma proteins, death from peritonitis or other infections, or a prolonged clinical course marked by repeated exacerbations and remissions, lipoid nephrosis becomes a rare disease and the kidneys at postmortem do not show the characteristic lesions of glomerulonephritis.

Even though we accept the restricted definition of lipoid nephrosis, we encounter many transition cases which cannot be distinguished from glomerulonephritis with certainty. The fact that we recognize a mixed type of nephritis and nephrosis (nephritis with nephrotic *Einschlag*) is an admission that the two diseases blend. The mixed type is particularly difficult to define clinically. How high a blood pressure or how much nitrogen retention is admissible to this group? Both hypercholesterolemia and the decrease of plasma proteins are more pronounced in lipoid nephrosis than in glomerulonephritis, but the difference is only in degree. A patient with lipoid nephrosis never develops uremia, but there may be a decreased elimination of phenolsulphonephthalein or a moderate nitrogen retention. The distinction based on renal function is also in degree only. In fact the two diseases blend in every clinical symptom and test. The clinical evi-

dence indicates strongly that lipoid nephrosis is not a distinct disease but a form of glomerulonephritis that does not tend to develop uremia.

Pathological Relation: The assumption that the glomeruli in lipoid nephrosis are practically normal is largely responsible for the view that the disease is entirely different from glomerulonephritis. When special stains are used which bring out the histological details of glomerular structure it is seen that definite lesions are present in every instance (see Table III for summary), but there is no uniform histological change characteristic of lipoid nephrosis.

Typical clinical glomerulonephritis shows uniformly in the acute stages a plugging of the capillaries with swollen endothelial cells, polymorphonuclear leucocytes and interlacing hyaline fibers (McGregor). In advanced stages the hyaline fibers and the basement membrane enlarge and fuse to give rise to a hyaline glomerulus. The histological features are, therefore: enlargement and increase in number of the endothelial cells, polymorphonuclear leucocytes, hyaline fibers and thickening of the basement membrane. In the 10 nephroses studied (Table III) there was a definite increase in the number and size of the endothelial cells in 8. In 8 of 9 cases in which the basement membrane was seen it was definitely thickened. The thickening was marked in four, and extreme in one instance. Hyaline fibers were found in the capillaries in three instances. Two of the cases showed small glomerular lesions indistinguishable from typical clinical glomerulonephritis, and a third case (Case X) corresponds closely with glomerulonephritis.

It may be said, therefore, that there are many points of resemblance in the glomerular structure of lipoid nephrosis and glomerulonephritis. The chief dissimilarity is that the glomerular capillaries are completely obstructed in glomerulonephritis and only partly obstructed in lipoid nephrosis. But a partial obstruction to the glomerular circulation has a very different effect on the subsequent macroscopic changes in the kidneys and the clinical course from that resulting from a complete obstruction. Complete obstruction produces hyaline glomeruli, atrophied tubules, contracted kidneys and finally uremia. Partial obstruction does not produce any of these four conditions, but the tubules become filled with lipoid, and the clinical course is that of lipoid nephrosis. Some years ago Löhlein called attention to the fact that a damaged glomerulus that

still functions is associated with a fatty tubule. Every case of chronic glomerulonephritis shows such glomeruli with fatty tubules (Fig. 13).

Lipoid nephrosis may therefore be regarded as a form of glomerulonephritis in which the glomerular capillaries are damaged but not completely obstructed. Even from this point of view it is still important to recognize typical cases clinically since it is a form of nephritis that does not terminate in uremia. The patient with this form of nephritis either recovers after a prolonged course or dies from an intercurrent infection.

No instance has come under my observation in which a patient with the clinical picture of lipoid nephrosis subsequently developed a contracted kidney.

Summary and Conclusions

1. A survey of recent literature shows a tendency to use "lipoid nephrosis" in a broad way to include all cases of nephritis with edema, albuminuria, normal blood pressure and normal blood nitrogen. This clinical definition includes many cases that are anatomically acute or mild chronic glomerulonephritis.

2. All the clinical phenomena of lipoid nephrosis, *viz.*, albuminuria, edema, normal blood pressure, hypercholesterolemia, decrease of the plasma proteins with reversal of the albumin:globulin ratio and normal blood nitrogen, may occur in a less pronounced form in glomerulonephritis.

3. There are many transition cases between lipoid nephrosis and glomerulonephritis, *viz.*, those that fit the definition of lipoid nephrosis except for the presence of hypertension or moderate nitrogen retention. These are called the mixed type, or nephritis with a nephrotic *Einschlag*. From the clinical standpoint the two diseases cannot be sharply separated.

4. A histological study has been made of 10 cases of large, fatty kidneys, without tubular atrophy, that were clinically nephritis. Four of these are classed as pure lipoid nephrosis, 3 as nephritis with nephrotic *Einschlag* (mixed type) and 3 as of uncertain type because of lack of clinical data.

5. The glomeruli are not normal in any instance, but the lesions are not of uniform type.

6. In pure lipoid nephrosis there is a varying increase in the number and size of the glomerular endothelial cells and an uneven thickening of the basement membrane.

7. In the mixed type (nephritis with nephrotic *Einschlag*) there is a marked thickening of the basement membrane. In one instance the capillaries were compressed by enlarged epithelial cells. In two instances there was a definite increase in the number and size of the glomerular endothelial cells, and a few areas in the glomeruli showed the changes characteristic of typical clinical glomerulonephritis, *i. e.*, capillaries filled with endothelial cells and a network of hyaline fibers.

8. Three large non-contracted kidneys were studied in which the data were insufficient to determine whether they should be classed as lipoid nephrosis. Two of these showed extreme thickening of the basement membrane. The third showed an enormous accumulation of fat in the tubules and glomeruli, large glomerular epithelial cells with hyaline and fatty degeneration, and leucocytes and hyaline fibers within the capillaries as in glomerulonephritis.

9. Lipoid nephrosis is to be regarded as a form of glomerulonephritis in which the glomeruli are damaged but their capillaries are only partially obstructed so that they continue to function and tubular atrophy does not occur.

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DESCRIPTION OF PLATES

PLATE 107

FIG. 1. Case I. A–28–647. Pure lipoid nephrosis. Hematoxylin-eosin stain. Photomicrograph. The capillaries are largely filled with mononuclear cells with a very pale vacuolated cytoplasm.

FIG. 5. Case VI. A–28–183. Mixed type — Nephritis with nephrotic *Einschlag*. Photomicrograph. Partial occlusion of glomerular capillaries by swollen endothelial cells. Diffuse tubular atrophy.

FIG. 7. Case VII. A–17–145. Photomicrograph. Adhesion of glomerulus to its capsule. The solid portion of the tuft at the site of the adhesion has the structure of typical glomerulonephritis.

FIG. 8. Case VIII. A–27–511. Renal lesion of uncertain type. Photomicrograph. Great increase in number of nuclei. Marked narrowing of the capillaries.



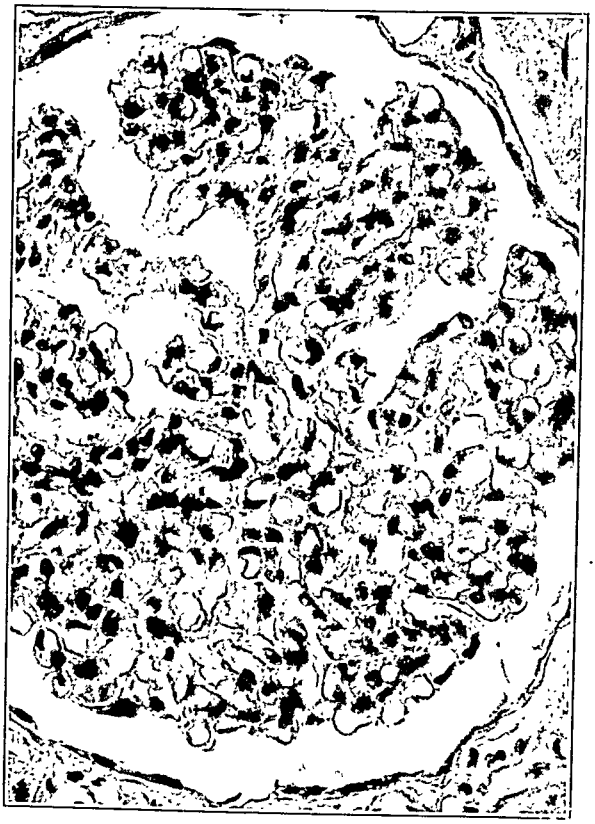
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Lipoid Nephrosis

PLATE 108

FIG. 2. Case I. A–28–647. Pure lipoid nephrosis. Mallory's anilin blue (Heidenhain, azan-carmine). Drawing of lobule of glomerulus (high magnification). The cells filling the capillaries are shown to be of endothelial origin. The vacuoles in the cytoplasm were occupied by lipoid droplets. The majority of the swollen cells (End. a.) are still firmly attached to the basement membrane, but a few are almost completely detached (End. f.) or entirely free. The basement membrane (B. M.) shows a moderate uneven thickening. The capillary lumina (L.) are markedly obstructed. The epithelial cells (Ep.) are inconspicuous.

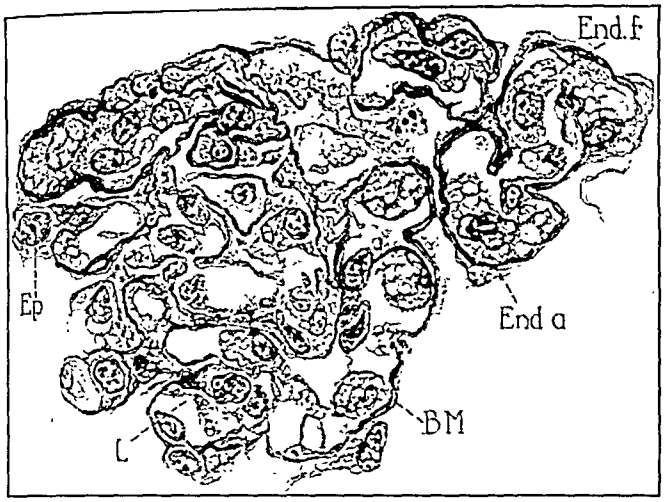
FIG. 3. Case II. A–28–5. Pure lipoid nephrosis. Mallory's anilin blue (Heidenhain azan-carmine). Drawing of part of a glomerulus (high magnification). A number of epithelial cells are seen (Ep.). There are many more endothelial nuclei (End.) than are seen in a normal glomerulus. One large endothelial cell is shown occluding a capillary (End. a.). The basement membrane (B. M.) is moderately thickened throughout, and in several places it shows very marked thickening (B. M. t.). There is partial occlusion of the lumina of some of the capillaries.

FIG. 4. Case V. A–28–193. Mixed type — Nephritis with nephrotic *Einschlag*. Drawing of part of a glomerulus. Stain as in Fig. 3. Prominent glomerular epithelium (Ep.). Increased number of endothelial nuclei. Thick basement membrane (B. M.). Ery., Erythrocytes.

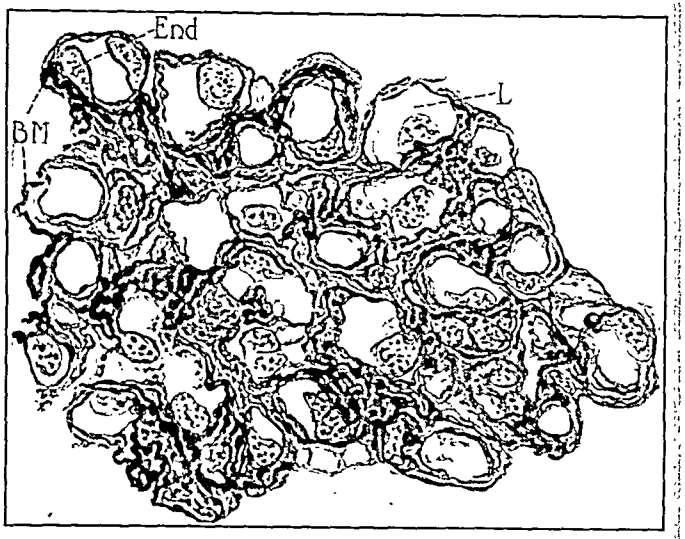
FIG. 6. Case VI. A–28–183. Mixed type — Nephritis with nephrotic *Einschlag*. Drawing of part of a glomerulus. Stain as in Fig. 3. Increased number of endothelial nuclei (End.). Marked thickening of basement membrane (B. M.). Polymorphonuclear leucocyte (P. M. N.). Ery., Erythrocyte; L., lumen of capillary.

FIG. 9. Case VIII. A–27–511. Renal lesion of uncertain type. Drawing of part of a glomerulus. Stain as in Fig. 3. Extreme thickening and duplication of the basement membrane (B. M.). Marked narrowing of lumina of capillaries (L.). Great increase of endothelial nuclei (End.).

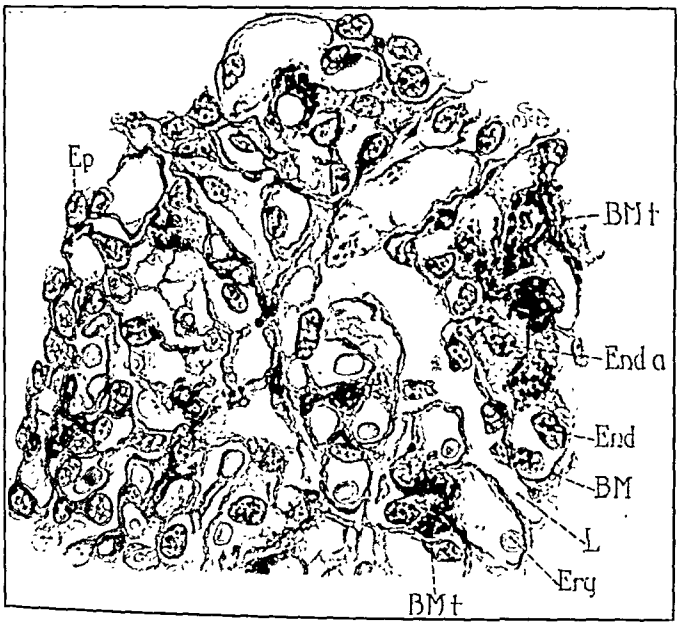
FIG. 11. Case IX. A–26–827. Renal lesion of uncertain type. Drawing of part of a glomerulus. Stain as in Fig. 3. The appearances are similar to Fig. 9, but the thickening of the basement membrane is more extreme, the lumina of the capillaries are smaller, and the endothelial nuclei are less conspicuous.



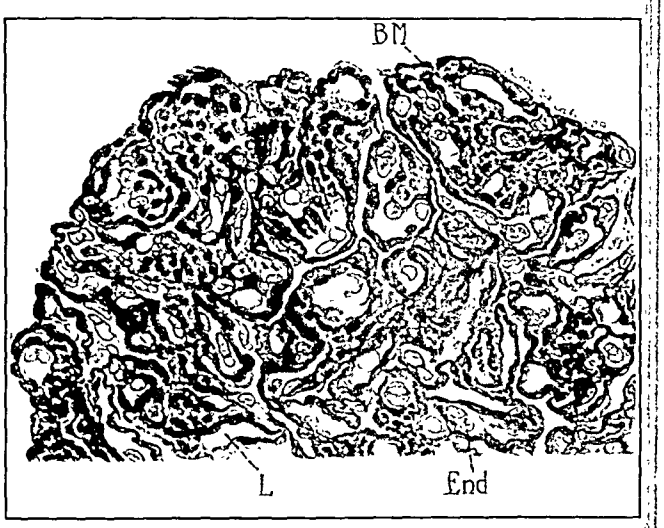
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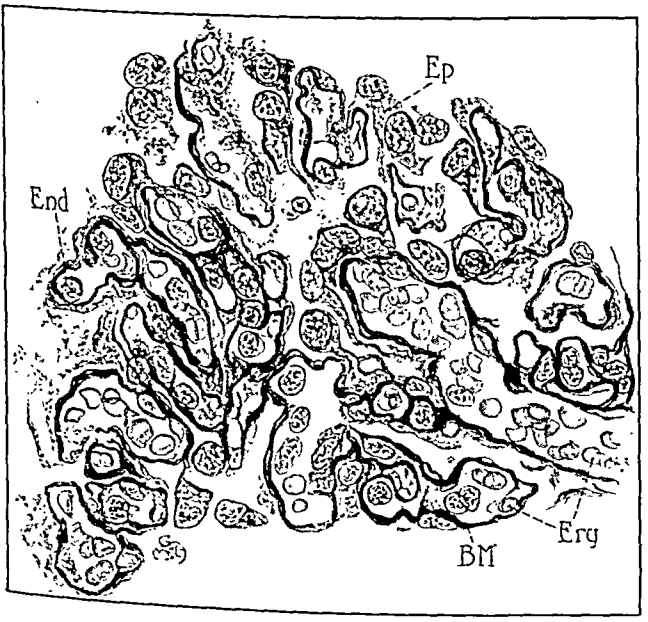
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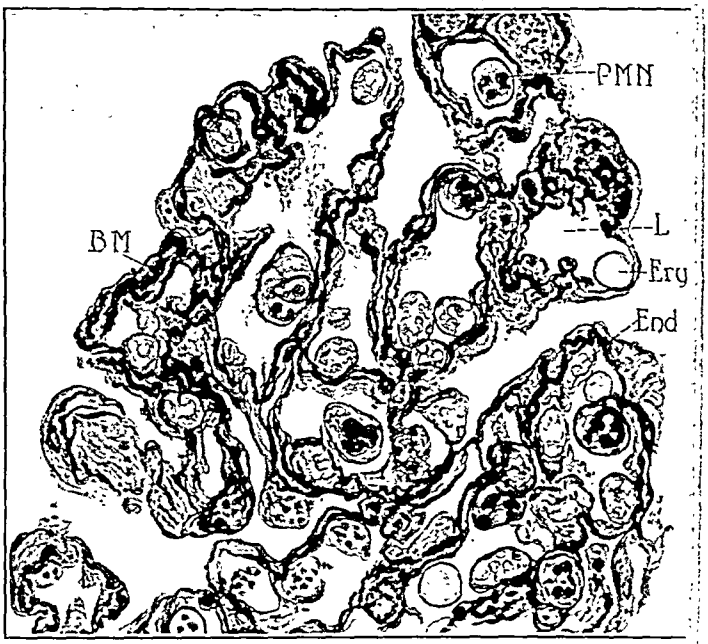


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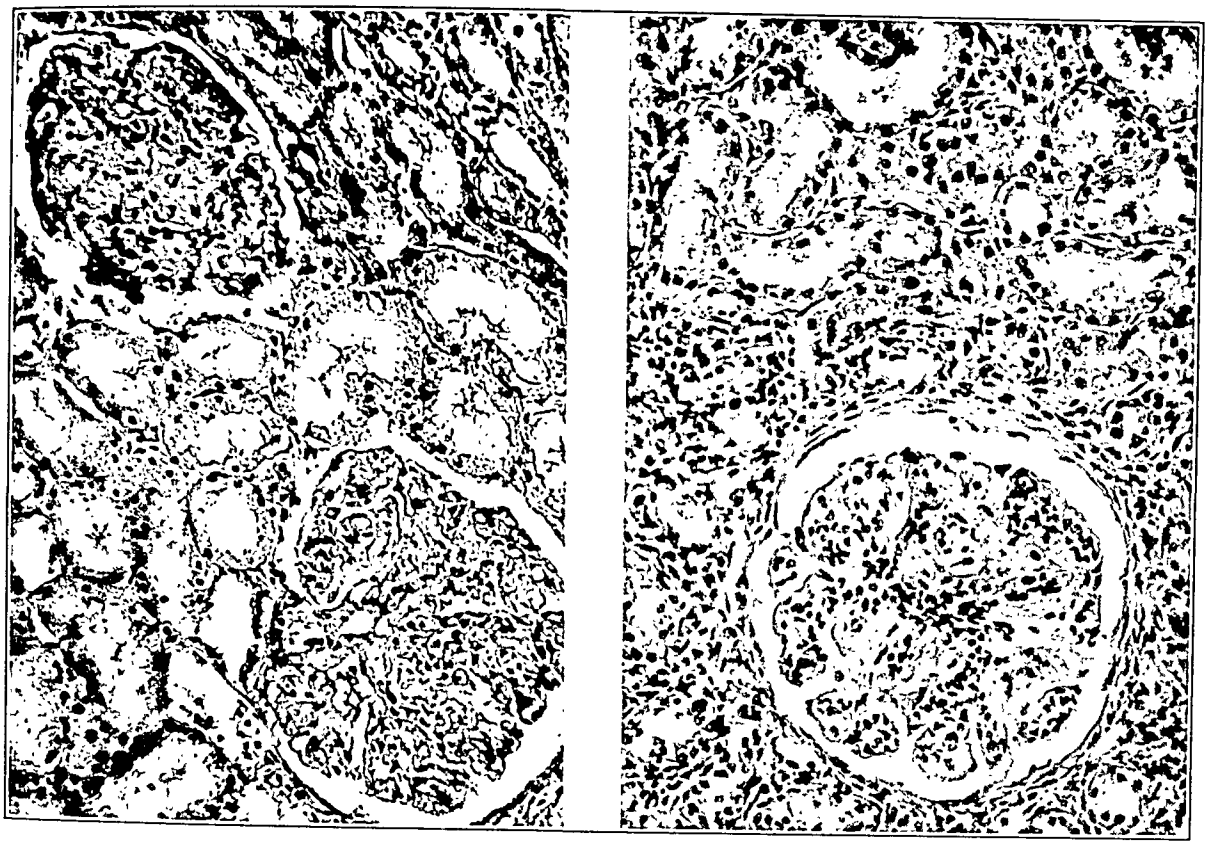
Lipoid Nephrosis

PLATE 109

FIG. 10. Case IX. A–26–827. Renal lesion of uncertain type. Photomicrograph. The glomeruli are of hyaline appearance, but the capillary bed is not completely obstructed. Large convoluted tubules.

FIG. 12. Case X. A–27–305. Renal lesion of uncertain type. Photomicrograph. Enormous enlargement of glomerular epithelial cells. Narrowing and occlusion of capillaries.

FIG. 13. Chronic glomerulonephritis. Photomicrograph. Glomerulus with its associated tubule. The capillaries are markedly narrowed but not completely occluded. The tubule is dilated, and its cells are filled with lipoid droplets.



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Lipoid Nephrosis

CARBON TETRACHLORIDE POISONING WITH MACROSCOPIC FAT IN THE PULMONARY ARTERY *

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Since the recent widespread use of carbon tetrachloride in the treatment of hookworm disease in animals and in man, our knowledge of the pharmacological and toxic effect of this drug is rapidly increasing.[1, 2, 3, 4, 5, 6] It is now established that the toxic manifestations are not due to impurities but that they are normal toxicological characteristics of carbon tetrachloride.[7, 8, 9] Clinical and experimental evidence indicate that the toxicity of carbon tetrachloride increases considerably when given with fatty substances or with alcohol, and in man most of the untoward reactions and fatal accidents occurred in chronic alcoholics who had taken alcohol before and after the administration of the drug.[10]

The symptoms of poisoning in man usually appear twelve to thirty-six hours after the ingestion of the drug, and these are characterized by nausea, vomiting, abdominal pain and tenderness. Later jaundice appears.

The most prominent histological changes are found in the liver, but the cortical cells of the adrenal as well as the kidneys are also involved, though to a less marked degree. The blood may be rich in bile pigments, but so far as we know no observations have been reported which show a high fat content of the blood as the result of carbon tetrachloride poisoning.

The case studied is of interest not alone on account of the completeness of the clinical and postmortem observations but also because of the high fat content of the blood and the unusual distribution of this fat in the vascular bed.

Report of Case

Clinical History: F. McG., a laborer, 34 years of age, was admitted in a stuporous condition to the Boston City Hospital, October 29, 1928. His chief complaints were pain in the abdomen and persistent nausea and vomiting.

* Received for publication July 8, 1929.

He has always been exceptionally strong and well, losing work only through periods of intoxication. During the last four years the patient has indulged in alcoholic debauches periodically. The most frequent beverages were "Noonan's Hair Petrol" and denatured alcohol. The patient was admitted to the hospital for one night, one week preceding his present entrance. After leaving the hospital he again drank for three days and on the morning of the third day he drank approximately one ounce of "Carbona" (carbon tetrachloride) in a glass of milk. The same evening he began to feel ill while at a friend's house, but was able to walk home. The following morning a physician was called because the patient suffered continuous abdominal pain, with hiccoughs, nausea and vomiting. The next day these symptoms had temporarily disappeared, but during the evening the pain and nausea recurred, associated with violent attacks of vomiting. The third day after ingesting carbon tetrachloride he became semicomatose and was removed to the hospital.

The family and past history were quite unessential and have little or no bearing on the present illness.

Physical Examination: When examined, he appeared to be well developed and nourished. He was lying quietly, resented any disturbance and was able to answer questions only occasionally. He frequently hiccoughed and vomited foul-smelling, dark fluid material suggesting old blood. His eyes and skin were moderately jaundiced. The skull and hair were normal. The pupils reacted to light but the reaction to accommodation could not be tested. The movements and fundi of the eyes were normal. The nostrils were filled with blood-stained exudate; the breath had a bad odor and the mouth and tongue were coated with a brownish material. The pharynx could not be observed because any attempt at examination induced vomiting.

The thorax was symmetrical and the respiratory expansions were normal. The liver dullness was at the fourth rib in front on the right, and at about the ninth thoracic vertebra behind on both sides. Resonance and breath sounds were normal.

The heart apex was felt in the fourth space in the nipple line. There was no apparent enlargement to percussion and the cardiac sounds were of fair quality and regular. The systolic blood pressure was 150 mm. of mercury and the diastolic 90.

The abdomen was much distended and tympanitic. Shifting dullness in the flanks and a fluid wave in the lower quadrants were both elicited. The liver edge was felt about two inches below the costal margin on the right. The other organs were not palpated since even slight manipulation seemed to induce vomiting. The genital organs were normal, and a rectal examination revealed no abnormal findings, though the patient frequently passed black fluid feces.

The extremities were equal and well developed, and the reflexes were normal.

At the time of admission the temperature was 101° F, which remained essentially unchanged. The heart rate ranged between 100 and 110, and the respirations rose to 30 terminally.

Laboratory Findings: Two specimens of urine showed traces of albumin. The white blood cell count was 13,700 cells per cmm., the red blood cell count 4,600,000 per cmm. with a hemoglobin content of 90 per cent; and the blood platelets were normal. The non-protein nitrogen at the day of death was 115 mg. per 100 cc. of blood. The Kahn test of the blood for syphilis was negative. The stools showed a four-plus guaiac test.

Progress Note: The day after admission the patient became still more uncomfortable and lapsed gradually into almost complete coma. The jaundice became more intense, cyanosis appeared and death followed on October 31, forty-eight hours after admission.

An autopsy was performed six hours postmortem.*

Autopsy Report

The body is that of a large, well developed and well nourished, deeply jaundiced, white, adult male.

Peritoneal Cavity: The surfaces are smooth and bile-stained. The pelvis contains about 15 cc. of reddish brown watery fluid. The diaphragm on the right and left is at the third and fourth intercostal spaces respectively. The lower margin of the liver is 2 cm. below the costal margin in the right midclavicular line and 4 cm. below the tip of the xiphoid process.

Pleural and Pericardial Cavities: Negative.

Heart: Weight 380 gm., is normal in size and contour. A few discrete petechial hemorrhages lie in the epicardium over the right auricle. The endocardium and valve leaflets are discolored greenish brown. Early atheromatous lesions may be followed along the coronary arteries.

Lungs: The lungs are moderately voluminous, heavy, edematous and congested. Scattered punctate hemorrhages are abundant beneath the pleura of both lower lobes. The fresh surfaces are dark red and exude frothy, blood-tinged, watery fluid. The mucosa of trachea and bronchi is deep maroon. The peribronchial lymph nodes are slightly enlarged, soft and unusually red.

Liver: Weight 2600 gm. This organ is uniformly enlarged, rather soft, smooth, greasy and opaque yellow. The fresh surfaces show in addition to the fat, considerable congestion which stands out as a fine red stippling against a yellow background.

Kidneys: Weight 400 gm. They are both enlarged and quite firm. The capsules strip spontaneously from smooth yellowish red surfaces. On section, the cortex is thickened, yellow and opaque and is sharply differentiated from the clearly defined and congested pyramids. The cortex varies from 0.8 to 1 cm. in thickness.

Adrenals: Negative.

* The autopsy was performed by Dr. T. Leary, to whom we are indebted for permission to report his findings.

Aorta: This is thin, smooth, elastic and bile-stained.

Alimentary Tract: There are a few superficial linear erosions along the lower end of the esophagus. A point at the lower end of one of these is surrounded by a small hemorrhage, and on washing away the blood, a tiny bleeding point is visible. The stomach contains about 500 cc. of dark brown fluid.

The Blood: The blood was separately collected and transferred into measuring cylinders from the pulmonary artery, right ventricle, vena cava inferior close to its entrance into the auricle, vena cava inferior close to the iliac veins, and from the pulmonary veins and left ventricle. The bile and blood-stained duodenal contents were also saved for examination.

Within a few minutes the yellow fat in the blood rose to the surface and solidified. It was surprising to observe that as high as 64 per cent of the total blood volume obtained from the pulmonary artery and 25 per cent of that obtained from the upper end of the inferior vena cava was pure fat. Specimens of blood from other sources showed no gross fat. While these figures show only approximate percentages because of the presence of clots in the venous system, they serve to demonstrate the high fat content of the blood in these vessels.

Report of microscopic Findings

Sections were fixed in Zenker's fluid, neutral formalin and alcohol-formalin, and embedded in both paraffin and celloidin. Hematoxylin and eosin, and eosin and methylene blue were the routine stains employed, while frozen sections of formalin-fixed material were stained with Sudan IV and Nile blue sulfate.

The most interesting lesions are found in the liver, lungs, brain, kidneys and adrenals. Unfortunately, early postmortem changes have occurred in many of the organs.

Liver: In the eosin-methylene blue preparations little more than a coarse reticulum framework coated by eosin-staining granular debris represents all that is left of the central and mid-zones of the lobules; here, the picture is more suggestive of lung tissue than normal liver. The extreme peripheral portions of the lobules are better preserved, and the cells bordering the portal connective tissue are more nearly normal and show signs of regeneration charac-

terized by an occasional mitosis. Cells of the outer zone, which lie more distant from the portal areas, are greatly distended with large and small clear vacuoles. This zone also shows a slight infiltration with endothelial and polymorphonuclear leucocytes. There are small clumps of alcoholic hyalin in the cytoplasm of a few of the liver cells.

In the frozen sections of the liver stained with Sudan IV, the central and mid-zones of the lobules are filled with large and small irregular confluent globules of fat. Nearer the periphery the fat droplets are more discrete, more uniform in size and are still within the cytoplasm of the liver cells. The hepatic veins are filled with fat, in contrast to the branches of the portal vein and hepatic artery which contain none. With Nile blue sulfate the greater portion of the fat stains pink, whereas only toward the periphery of the lobule does it stain blue, and here it appears as fine and coarse granules.

Trachea and Lungs: The epithelium has largely desquamated from the surfaces of the trachea and larger bronchi. The underlying vessels are congested and the submucous connective tissue is infiltrated with serum, fibrin and red blood cells. Here and there, small groups of alveoli are filled with freshly extravasated blood.

Sections of varying thickness stained with Sudan IV show that while the larger branches of the pulmonary artery contain masses of fat, many of the smaller branches are completely filled; the alveolar capillaries contain but rarely minute fat droplets. There is no visible fat in the alveolar spaces, even in the areas of hemorrhage, nor are there areas of infarction necrosis.

Kidneys: Both the glomerular capsules and tubules contain precipitated albumin, while the tubules in addition contain homogeneous and coarsely granular eosin-staining casts, a few desquamated epithelial cells and an occasional endothelial leucocyte. The tubular epithelium is granular and shows a rare mitotic figure.

When stained for fat, small fat cylinders are visible within the glomerular capillaries, and fatty casts are present in the collecting tubules. The cytoplasm of the cells lining both the convoluted and collecting tubules is filled with small fat droplets.

Brain: Sections of the brain embedded in paraffin and stained with eosin-methylene blue show no pathological lesions. In sections stained with Sudan IV, small fat droplets are quite commonly seen within the capillaries.

Adrenals: Apart from an occasional mitotic figure among the cells of the cortex, there is no other pathological finding.

Esophagus: The epithelium is desquamated and the vessels are everywhere congested. Lymphocytes, endothelial and polymorphonuclear leucocytes and red blood cells infiltrate the submucous connective tissue.

Microscopic Diagnoses: Alcoholic cirrhosis (early); toxic hepatitis; visible fat in vessels of the liver, lung, brain and kidney; tubular nephritis with fatty degeneration of the tubular epithelium; hemorrhages into the submucosa of the trachea and bronchi, and also into the alveolar spaces of the lungs; toxic reaction of the adrenals, and superficial ulceration of the lower end of the esophagus.

DISCUSSION

The most interesting pathological lesions are in the liver, and the study of this organ may largely explain the unusual fatty state of the blood seen at the time of the autopsy. Carbon tetrachloride, in large amounts, is known to exert a distinctly toxic action on the liver, and simulating chloroform in its action it first produces degenerative changes in the cells of the central zones which later extend through the mid-zone into the periphery of the lobule. In small amounts, the liver cells in the central and mid-zones may show simply fatty degeneration, whereas when large quantities are absorbed it causes rapid degeneration and necrosis.

This case is probably best explained on the basis that the patient had a large fatty liver of the type sometimes seen in early alcoholic cirrhosis. The carbon tetrachloride caused degeneration and necrosis of the cells of the central and mid-zones allowing the individual fat droplets to coalesce into larger and irregular masses. This liberated fat gained an easy access to the hepatic system of veins and consequently was carried along into the right side of the heart and out into the pulmonary circulation.

The fact that only a negligible amount of the fat reached the left side of the heart was probably due to the large size of the fat globules, and this also explains why the fat gradually increased in amount in the larger pulmonary arteries.

The small hemorrhages in the epicardium, the connective tissue of the mediastinum, beneath the serosa of the jejunum, and in the

lungs, may be explained either on the basis of an acute cholemia, or an acute toxemia; in the case of the lungs, however, the possibility of fat emboli must also be considered.

In the systemic circulation, fat was seen in the vessels of nearly all the organs as very minute droplets. These minute droplets may have reached these organs as a result of their passage through the pulmonary capillaries, or may have formed in the blood from still smaller droplets, due to the action of carbon tetrachloride on very minute fat globules present in the circulation. Although a few of the capillaries in the brain and in the glomerular tufts of the kidneys revealed even larger droplets which seemed to occlude the lumina, there were no visible embolic lesions.

The superficial ulcerations occurring along the folds of the lower end of the esophagus in cases of chronic alcoholism associated with vomiting have been described recently by Mallory and Weiss.[11] They are considered to be of mechanical and chemical origin and may be the site of fatal hemorrhages.

Summary

1. The gross and microscopic findings are described in an unusual case of carbon tetrachloride poisoning in which the blood in the right side of the heart and larger pulmonary arteries contained an extremely high percentage of fat (60 per cent).

2. The severe damage to the liver, which was already large and filled with fat, was the probable source of the fat in the vascular system.

3. Fat droplets that were small enough to pass through the capillaries of the lung and to gain entrance into the capillaries of other organs produced no microscopic lesions in either the heart, brain or kidney.

4. It appears from these observations that the sensitiveness of alcoholic patients to carbon tetrachloride is increased not only because of increased absorption of the drug, as well as the synergistic action of alcohol and carbon tetrachloride together in producing liver damage, but also because of the possible presence of preëxisting liver damage.

We are indebted to Dr. F. B. Mallory for the photomicrographs, and to Miss Marion Lamb for technical assistance.

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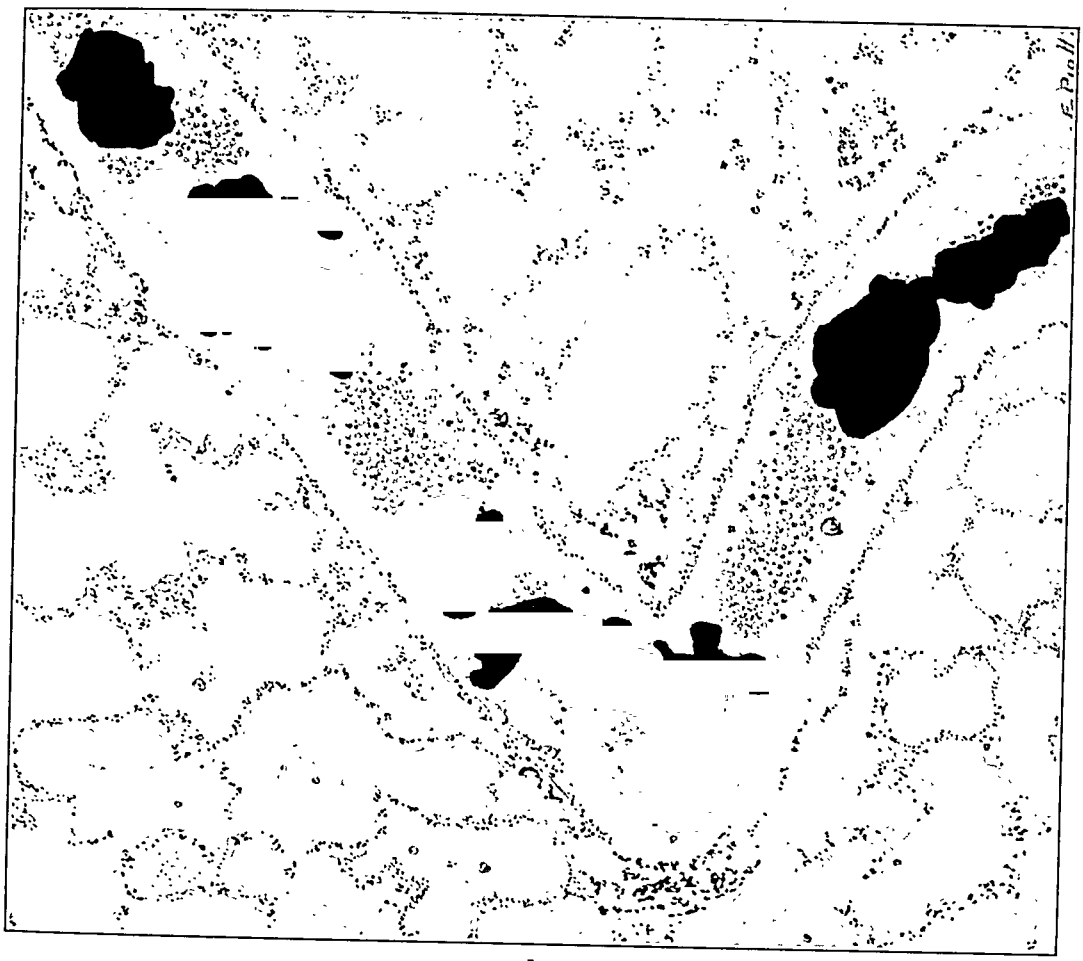
DESCRIPTION OF PLATES

PLATE 110

FIG. 1. Low power drawing of the lung, stained with osmic acid, showing several large, irregular fat globules nearly filling one of the smaller branches of the pulmonary artery. Scattered endothelial leucocytes containing carbon pigment lie in the adjacent stroma.

Fig. 2. Photomicrograph of an area of the liver from a typical case of fatty alcoholic cirrhosis. The fat droplets are regular in distribution and of a fairly uniform size. Sudan IV. × 250

FIG. 3. Photomicrograph of an area from the liver of the case reported. The contrast between this and Fig. 2 is quite marked. The fat is irregular in distribution and is very uneven in the size of globules. Sudan IV. × 250.



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MacMahon and Weiss



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Carbon Tetrachloride Poisoning

PLATE III

FIG. 4. Blood removed from the pulmonary artery, showing about 60 per cent fat.

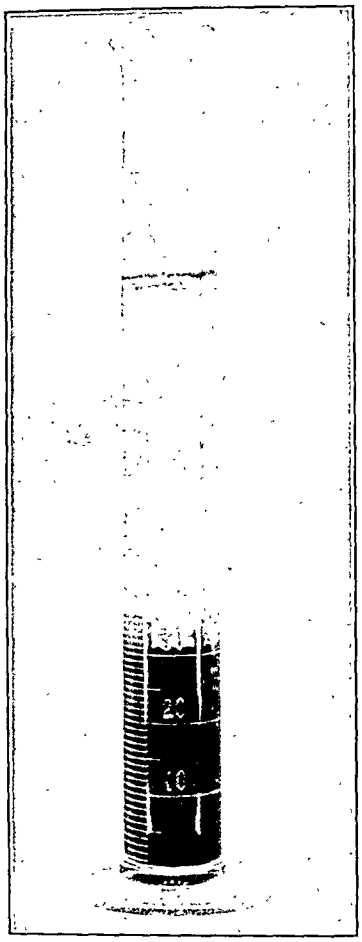
FIG. 5. Blood removed from the pulmonary vein, showing no visible fat.

FIG. 6. Blood removed from the upper portion of the inferior vena cava showing about 25 per cent fat.

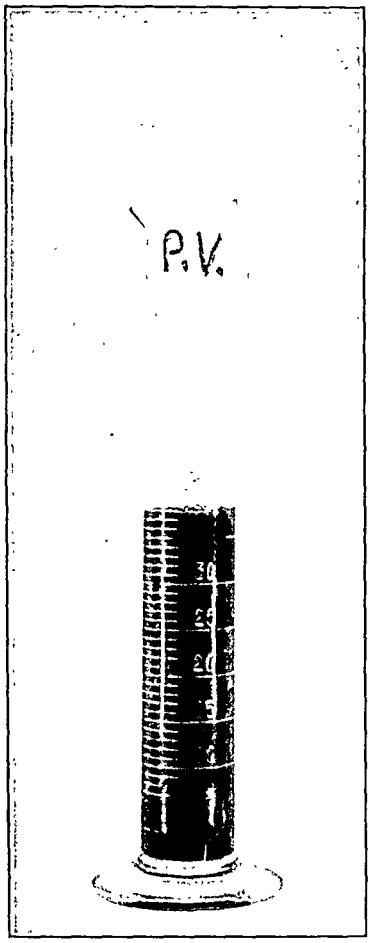
FIG. 7. Blood removed from the lower portion of the inferior vena cava. The amount of gross fat is negligible.

FIG. 8. Specimen of bile removed from the gall bladder, showing no visible fat.

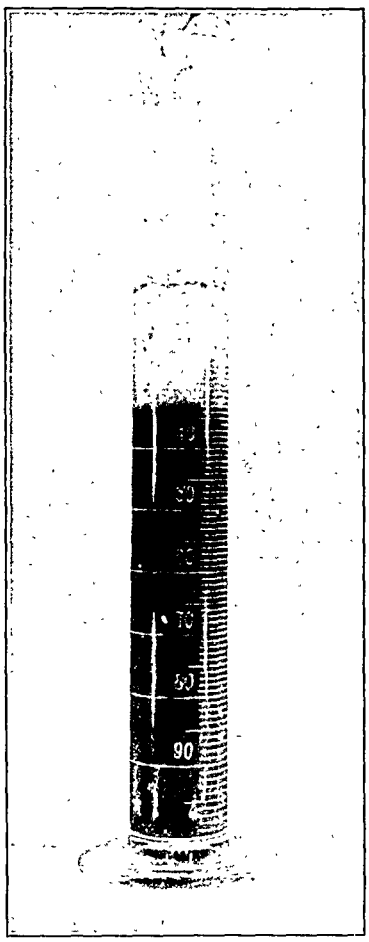
FIG. 9. Blood-stained contents of the duodenum and jejunum, showing a narrow rim of fat along the surface.



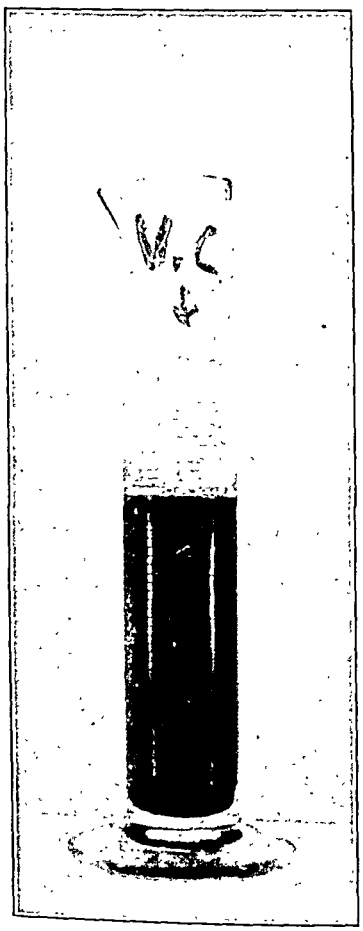
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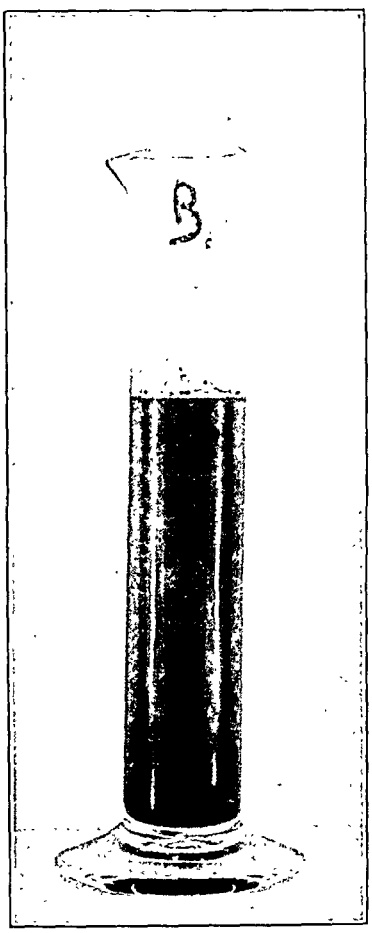
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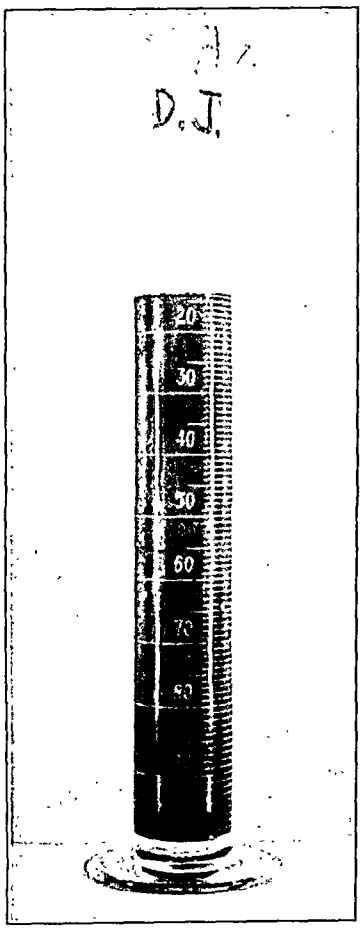
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MacMahon and Weiss

Carbon Tetrachloride Poisoning

EXPERIMENTAL OBSTRUCTIVE CIRRHOSIS *

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The term "obstructive cirrhosis" has been chosen to indicate that type of cirrhosis which occurs in a liver simply as the result of prolonged obstruction to the outflow of bile. The term "biliary cirrhosis" has been avoided because it has come to be used in a rather loose manner to indicate any form of cirrhosis which is confined largely to the portal areas, irrespective of the cause.

Already a great deal of work has been done in this field of pathology, much of which was carried out years before aseptic precautions were observed in experimental surgery. And yet, in spite of the rather crude surgical technique of earlier workers, and in spite of infections, hemorrhages and biliary fistulae, the results obtained sixty years ago were not essentially different from those which have been reported in recent years.

Literature

In the year 1866, Leyden [1] and Wyss [2] working independently, reported their findings in dogs following obstruction of the common duct. Many of their animals became infected and died early; a few, however, that lived longer showed lesions which were described as biliary cirrhosis of the liver. Mayer,[3] six years later, repeated their work and in the few animals that survived he found similar lesions. In 1873, Legg [4] reported the microscopic lesions found in the livers of cats following ligature of the common duct, and according to this worker, "the most important change is an increase in connective tissue which can be made out within a few hours, and which progressively increases destroying the tissue of the liver before it."

* Received for publication July 8, 1929.

In almost every succeeding year a paper has appeared dealing with this problem in pathology; however, reference will be made here only to those papers which discuss the histological changes as they are found in the liver of the guinea pig and other mammals.

Charcot and Gombault [5] in 1876 described three separate lesions; first, areas of necrosis; second, an increase in connective tissue confined largely to the interlobular areas; and lastly, an increase in bile ducts. Chambard,[6] a year later, verified these findings and in addition found mucous droplets within the cytoplasm of the liver cells and an interlobular increase in connective tissue, an observation later substantiated by Beloussow.[7] Litten [8] and Steinhaus [9] reported similar lesions except that they were unable to find an appreciable increase in connective tissue, whereas Bauer,[10] writing in 1882, considered the increase in connective tissue to be the outstanding feature. Lahousse,[11] differing from all earlier writers, found solely an increase in connective tissue without any change in the bile ducts.

Harley [12, 13] in 1898, and later in 1901, was one of the first to adopt an aseptic technique in the ligation of one hepatic duct in dogs and cats. He gave no bacteriological report but described a form of cirrhosis characterized by an interlobular fibrosis, a dilatation of the ducts and an atrophy of the liver cells.

Jagić,[14] Tischner,[15] and later Richardson [16] in 1911, and Rous and Larimore [17] in 1920 ligated the bile ducts of rabbits and found lesions essentially similar to those previously reported by Charcot and Gombault.

Ogata [18] took up a similar study in guinea pigs. He ligated the common duct in four animals; the first died in forty-eight hours with generalized peritonitis; the second, after the same length of time, showed no appreciable gross lesions; the third and fourth died suddenly on the fifth and eighteenth days respectively as a result of perforated gall bladders. His findings warrant a more detailed description although the possibility of infection and free bile in the peritoneal cavity must be considered as possible additional factors responsible for the histological lesions he described. After forty-eight hours obstruction he found typical areas of necrosis confined largely to the periphery of the lobules, scattered mitoses in the liver cells, and dilatation of the interlobular bile ducts. At the end of the fifth day, the areas of necrosis were bordered by encroaching

granulation tissue, the connective tissue had increased in the portal areas, and there was a new growth of bile ducts, of which only a few made connections with the liver cells. At the eighteenth day, the lobules were reduced to one-half their normal size by the increase in bile ducts and connective tissue. The new bile ducts were described as being very tortuous and irregular, and the many new sprouts extending out into the connective tissue resembled branches of a tree. At this later stage the necrotic areas were much less numerous.

Foà and Salvioli [19] in 1877 called attention to the fact that various experimental animals react differently following ligature of the common duct. This was an important observation for it must be considered in interpreting the apparently contrary conclusions arrived at by various workers using different animal species.

Ogata carried out a series of experiments similar to those performed in guinea pigs, using rats, rabbits, dogs, mice, pigeons and frogs, and found the usual necrotic areas in all animals except the dogs, frogs and mice; in the mice, however, the period of post-operative survival was but a few hours. There was a prominent and early increase in bile ducts in the rats and rabbits, whereas there was no such finding in either the pigeons, frogs or in a dog that lived but a week; however, the number of bile ducts was slightly increased in a dog that survived for thirty-five days. An increase in connective tissue was observed in all the animals which showed an increase in bile ducts.

From a review of this wide group of experiments it is apparent that as a result of prolonged bile stasis the liver does show definite changes that vary somewhat in different animals, both in respect to the histological findings and in the rapidity with which they are produced. However, in spite of the fact that almost identical lesions have been observed by many workers in different countries and at different times, there is no general agreement in either the interpretation or explanation of their findings.

Report of Experimental Work

Large healthy male guinea pigs were used because in these animals the lesions appear early, infection is infrequent, and the period of survival is sufficiently long to permit the changes becoming well advanced.

With the animals etherized, the peritoneal cavity was opened; the common duct was carefully isolated and ligated just at its entrance into the duodenum. The animals were afterwards kept separately and given their usual diet of greens, grain and water.

In an attempt to study the earliest changes in the liver, and to follow the lesions from day to day, the first animals to be examined were autopsied twelve hours after the operation, while others were autopsied every twenty-four hours thereafter for a period of twelve days. At the time of the autopsy, cultures were taken from the peritoneal cavity and liver and grown both aerobically and anaerobically. Moreover, only those animals were included in which there was no obvious infection, hemorrhage or free bile within the peritoneal cavity, and lastly, in which all cultures were sterile.

To obtain good fixation of tissue, the animals were bled at the time of the autopsy and pieces of tissue were placed immediately in formalin and in Zenker's solutions. The gall bladder in some of the animals was opened and the liver sectioned immediately; in others, the biliary system was preserved intact, in which case the portal vein was perfused with saline followed by Zenker's solution. In others, the portal vein was injected with India ink and the liver was preserved *in toto*, while in still others, a rather unsuccessful attempt was made to replace the bile and distend the biliary system with a suspension of ink in agar. To ensure that ligation had been complete, the duodenum was opened and pressure was applied to the gall bladder and common duct above the ligature, while an attempt was made to pass a small probe from the ampulla of Vater beyond the point of constriction.

Sections were embedded both in paraffin and in celloidin and stained with eosin and methylene blue and hematoxylin and eosin, while frozen sections of formalin-fixed material were stained with Sudan IV and Nile blue sulfate.

PATHOLOGICAL REPORT

This is based on a total of twenty "test" animals in which the period of survival after ligation of the common duct ranged from twelve hours to twelve days and is confined to an examination of the liver and extrahepatic biliary system.

The most outstanding lesion was the marked distention of the extrahepatic biliary system, which was observed as early as twelve

hours and found of even a greater degree in the animals that survived longest. In two of the animals which have not been included in the series, the gall bladder had ruptured, a finding not infrequently reported by former workers.

The liver becomes slightly enlarged in from twenty-four to forty-eight hours after ligation. At this time its surface is smooth and the capsule seems distended; later, after eight, ten and twelve days, the surface is very finely stippled, not unlike fine Morocco leather. The liver is bile-stained, but the color, unlike that caused by human bile, appears yellowish brown. Small, rather irregular, yellowish white areas, varying from 1 to 3 mm. in diameter are distributed beneath the capsule of the liver. At first there is no irregularity in the capsule over these anemic areas, but after several days the smooth capsular surface shows a very shallow pitting.

During the first few days there is no appreciable change in the consistence of the liver but later it becomes firmer and offers more resistance to cutting. When a liver is sectioned before fixation, watery yellow bile flows freely from the dilated ducts, the organ shrinks slightly and the ducts become partially collapsed. If, however, the liver is fixed before sectioning, the ducts and vessels remain distended even though the bile mostly escapes. When the portal vein was perfused with ink, the only portions of the liver that did not become discolored were the small scattered areas of necrosis.

Microscopic Report

Large Bile Ducts: The earliest and perhaps most important change to appear, and one that probably begins as soon as the common duct is ligated, is a gradual and progressive dilatation of both the extra- and intrahepatic biliary systems. With the gradual distention of the ducts, the epithelium at times becomes definitely flattened, and mitoses are quite numerous. Both the surrounding fibroblasts and liver cells show signs of this expansion; they are stretched, elongated and more compact than usual.

Areas of Necrosis: Great emphasis has always been given to large and small areas of necrosis that are commonly found in the periphery of the lobule. Because they occur very early and form rather outstanding histological lesions they are described here; however, they are of slight importance. In the twelve-hour guinea pig these

areas of necrosis are scattered throughout the entire parenchyma. They vary in size, some involving only a few cells, others an entire lobule. The liver cell is the first to show degenerative changes; later the endothelium lining the sinusoids; lastly, the reticulum framework, and when the lesion is centered about a small portal area the blood vessel is thrombosed, its wall necrotic, and even the small terminal bile duct epithelium may show both cytoplasmic and nuclear degeneration. A few of these areas contain scattered extravasated red blood cells, usually at the periphery. The most recent necrotic areas show no cellular reaction, a little later polymorphonuclear leucocytes are seen at the periphery, whereas after twenty-four to forty-eight hours the margin is infiltrated with both polymorphonuclear and endothelial leucocytes.

These areas of necrosis are formed largely within the first twenty-four hours after ligation; however, new areas do appear even up to twelve days. They do not seem to increase in size. On the contrary, those which are several days old are smaller, the adjacent liver cells have proliferated and a dense infiltration of endothelial leucocytes bordering the periphery have phagocytosed and removed much of the necrotic cellular débris.

Terminal Bile Ducts: The first change to occur in the bile ducts, apart from the simple dilatation, is a proliferation of the bile duct epithelium. This is shown by the presence of occasional mitoses as early as twenty-four hours. In forty-eight hours the bile ducts are already unusually prominent and mitoses are very numerous. On the third day bile is sufficiently concentrated in the distended ducts to become easily visible, and the bile duct epithelium shows still greater proliferation. With each successive day there is continued proliferation of the bile duct epithelium although after the tenth day mitoses are less numerous. With this great proliferation of the bile duct cells there is a marked elongation of preëxisting bile ducts with an apparent increase in their number. The terminal bile ducts of a normal guinea pig's liver are not always within the portal area; frequently they are seen traversing the peripheral zone of the lobule. This is important in interpreting the lesion sometimes seen in the peripheral zones during the later stages of biliary obstruction, because these apparent aberrant ducts, like the ducts lying within the portal areas, elongate, become tortuous, coiled and much more prominent. With this apparent increase, elongation and tortuosity

of the bile ducts, the portal areas are enlarged, they link up one with another dividing the liver parenchyma into separate and discrete lobules about two-thirds of the normal size.

Bile Capillaries: The bile capillaries lying between the liver cells are followed with difficulty during the first few days of obstruction, but later they become more clearly defined. A finding that is quite different from the picture seen in the human liver during biliary obstruction is the marked dilatation of that portion of the bile capillary adjacent to the portal area. In this area, the bile capillaries are distended into small cyst-like spaces with the bordering liver cells so flattened that it is difficult to distinguish them from proliferating bile duct epithelium.

Connective Tissue: The connective tissue in the portal areas is not appreciably increased until about the sixth day, although mitoses are found occasionally in fibroblasts as early as the third day. In the early stage this proliferation and increase in connective tissue is a relatively less prominent feature than the apparent increase in bile ducts, whereas later, in the ten and twelve-day animals, both bile ducts and connective tissue appear equally conspicuous. Moreover, resembling in distribution the dilated and elongated bile ducts, the connective tissue is confined to the interlobular areas.

The Liver Cells: As early as twelve hours the liver cells in the central and mid-zones are swollen, the cytoplasm is finely granular and contains small droplets of bile; later, hydropic degeneration of the liver cells is a more common finding and binucleate cells are frequent. Occasional individual cells scattered equally throughout different portions of the lobules show degeneration and necrosis. About the third day these necrotic cells become more plentiful and are seen more frequently adjacent to the portal areas and throughout the peripheral zone. Later these necrotic liver cells become less numerous and in the ten and twelve-day animals they are very infrequent. At the same time that one sees necrotic cells, other nearby cells show mitoses, a few as early as twenty-four hours, but after the second and third day, as many as one or two are seen in each oil immersion field. This proliferation of the liver cells decreases in the later stages so that in the twelve-day animals mitoses in the liver cells are practically absent.

The Kupffer Cells: Very early the endothelial cells lining the sinusoids are swollen and vacuolated. Some contain fat droplets, others

are distended with granules of hemosiderin. Mitoses are first seen in forty-eight hours; they become more numerous for a day or two and later almost disappear. With distention of the bile capillaries and swelling of the liver cells the capillary sinusoids are narrowed and often difficult to follow.

Cells in Portal Areas: An infiltration of cells into the portal areas appears in twenty-four hours and increases up to about the sixth or eighth day. Endothelial leucocytes are the first to appear; they proliferate rapidly and constitute the greater proportion of the free cells. Lymphocytes are less abundant, while scattered eosinophiles and occasional neutrophiles, though much less numerous, are seen in almost every portal area.

Blood Vessels: The portal veins, especially the medium-sized and larger vessels, become greatly distended. Usually the walls are quite normal, however small vessels with necrotic walls and lumina filled with fibrin thrombi are seen either in or bordering the areas of necrosis.

Lymphatics: In two of the test animals some of the larger lymphatics that drain the liver were included in the ligature of the common duct with the result that the lymphatics traversing the portal areas are extremely distended. Usually, however, these vessels are only slightly dilated and consequently much less conspicuous. Early, the portal connective tissue is unusually edematous but in the later stages this largely disappears.

Summary of Histological Findings

In reviewing the histological findings, it is apparent that as a result of biliary obstruction definite changes take place in the liver involving not only the large and small bile ducts but also the liver cells, the connective tissue stroma, capillary sinusoids and blood vessels. In the early stages the picture is characterized largely by the changes within the liver cells, the capillary bile stasis, the areas of necrosis and the cellular infiltration into the portal areas, whereas later it is dominated largely by the proliferation of bile duct epithelium, the elongation, tortuosity and apparent increase in bile ducts, and the increase in connective tissue in the portal areas. The end result is a rather uniform type of cirrhosis limited to the portal areas in which the lobules are diminished in size as a result of the expanding portal areas.

Discussion

Former workers have been unanimous in describing a proliferation and increase in the bile ducts, while Ogata even states that these new bile ducts frequently fail to make complete functional connections with the bile capillary system. Furthermore, in respect to the origin of these ducts Pick [20] believes that some arise from already existing bile ducts while others arise from the liver cells themselves. Gerhardt substantiates this view but Ogata could find no proof that liver cells could undergo such a transformation. From our studies we believe that the increase in bile ducts is simply an apparent change resulting from a proliferation of bile duct epithelium. In the livers that were fixed *in toto* with the bile ducts fully distended, we were able to follow the lumina of the elongated terminal bile ducts to their connections with the dilated lumina of the bile capillaries. Furthermore, India ink that was injected into the common duct could be followed through the tortuous terminal bile ducts into the bile capillaries.

Regarding the origin of the cells that form this apparent increase of bile ducts, we favor the opinion expressed by Ogata that they arise purely from a multiplication of preëxisting bile duct cells; however, in the later stages, when the liver cells lining the distended bile capillaries so nearly simulate bile duct epithelium it is almost impossible to differentiate the two types of cells and hence the embryonic or liver cell origin of bile duct epithelium cannot be denied positively.

The cause of the proliferation of the bile duct epithelium has also been a debated question, largely because of the misinterpretation of the existence of "new bile ducts." Chambard, Foà and Salvioli, Simmonds [21] and Pick explained the apparent increase in bile ducts simply as a concomitant phenomenon or as a sequence to the increase in connective tissue. Charcot and Gombault, and later Gerhardt, took the viewpoint that the bile duct proliferation was not merely a natural accompaniment to the connective tissue proliferation, but rather a separate and distinct process resulting from the increased pressure within the walls. However, Gerhardt also granted that an atypical growth of bile ducts could result from an increase in connective tissue. According to Ogata the bile duct and connective tissue proliferation are two quite unrelated processes re-

sulting from a thorough saturation with retained bile. It would appear, however, that since we are not dealing with a new formation of bile ducts but simply with an elongation of the connections that already exist, the presence of bile within the bile ducts would seem to be a sufficient and satisfying cause. Furthermore, the increase in connective tissue that is present in the portal areas has most likely been caused by a similar mechanical process of compression and stretching.

Much discussion has always arisen in regard to the origin of the areas of necrosis. Charcot and Gombault held the opinion that the pressure of retained bile ruptured the smaller bile ducts with the result that bile escaped into the lobules and killed the liver cells. Beloussow, Eppinger,[22] Abramow and Samoilowicz,[23] and Janowski,[24] from observations on human cases adopted a similar view. Foà and Salvioli found no rent in the bile ducts and thought that the increased pressure within the bile capillaries was sufficient to explain this necrotic process. Gerhardt, who also found no break in the biliary system, considered that the necrosis was the result of the chemical action of stagnant bile. Richardson and Ogata likewise considered that the injurious action of the bile was the cause of necrosis. Ogata, in attempting to explain the distribution of the necrotic areas, formulated the hypothesis that since all liver cells are not working at an equal rate at the same time and also because the outflow of bile in all capillaries is not equally rapid, then any sudden obstruction to the bile would result in a molecular destruction of the most actively functioning elements.

Tischner, years earlier when working with rabbits, felt that the degree of functional activity of the liver cells might play some part in determining whether or not they would undergo degeneration and necrosis, yet he considered it a relatively unimportant factor. He explained the lesions as simply areas of anemic necrosis resulting from obstruction to the circulation. The latter he estimated to be definitely decreased as a result of the increased pressure within the biliary system.

From our own observations we feel that the toxic effect of the bile, whether it reaches the liver cells by leakage or seepage, is of little importance. If it were extremely toxic the liver cells in the central zones that are filled with it from the onset would presumably be the first to undergo necrosis. These areas of necrosis resemble infarcts

in their appearance, contour and position, also in the type of inflammatory reaction that is limited to the periphery, and lastly in the manner of healing.

The effect of ligating the common duct is first to distend the bile ducts and produce a rise in pressure within the biliary system. Coincident and dependent on this is a probable decrease in the blood flow through the liver. Now, in addition to this general impairment in the hepatic circulation, there are the local changes which affect the terminal vessels. With distention of the bile ducts these smaller vessels may be collapsed entirely. This prevents the blood from flowing into the lobules and results in the formation of infarcts.

That these infarcts occur more commonly in the guinea pig and rabbit than in the dog and man is probably to be explained on an anatomical and physiological basis. The circulatory system is different; in man even the smallest portal areas contain both a branch of the hepatic artery and portal vein, whereas in the lower animals similar areas contain but a single, small, thin-walled vessel. This means that the most distal lobules are supplied with vessels that correspond to "end arteries." Furthermore, this implies that the connection between the hepatic artery and portal vein is not alone through a network of capillaries and sinusoids but also by larger communicating vessels, a fact which may be readily observed when India ink is injected into either the arterial or venous system.

The relation between the terminal vessels and bile ducts in guinea pigs is also rather unusual. They lie side by side, held together by a few strands of connective tissue which separate them from the surrounding liver cells. With such a close relationship and in such a restricted space, any distention of bile duct would of necessity encroach upon the vessel.

There is another point that is probably as important as either of these anatomical peculiarities and that is the amount of bile that is secreted by the different species of animals, for both the guinea pig and rabbit produce relatively many times as much bile as either dog or man.

Considering these three factors together, namely, the presence of "end arteries," the intimate relationship of vessels and bile ducts, and lastly the large quantity of bile secreted, it seems reasonable to assume that they would constitute a sufficient cause for the more frequent occurrence of infarcts in this group of lower mammals.

SUMMARY

Obstructive cirrhosis may be defined as a characteristic form of cirrhosis uniformly limited to all the portal areas throughout the liver. It is caused by an obstruction to the outflow of bile. In the early stages, bile stasis, necrosis and regeneration of individual liver cells, edema and infiltration of the portal areas are the most prominent features. Later there is an apparent increase in the number of bile ducts, the portal connective tissue is increased and with enlargement of the portal areas the liver becomes divided into small, separate and distinct lobules. In the guinea pig and rabbit, large and small areas of necrosis are early and constant findings.

Evidence is presented to show that the proliferation of bile duct epithelium results merely in an apparent increase in the number of bile ducts in the portal area and not in an absolute increase as has been described previously.

The proliferation of fibroblasts in the portal areas is interpreted on a mechanical rather than on a toxic basis.

The origin of the areas of necrosis is discussed, and the opinion is expressed that they are simply areas of infarction. Their relative frequency in lower mammals may be explained on an anatomical and physiological basis.

We are indebted to Dr. F. B. Mallory for his many suggestions and helpful criticism, and also for the photomicrographs. We also wish to thank Miss Marion E. Lamb and Miss Vida Randolph for technical assistance.

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DESCRIPTION OF PLATES

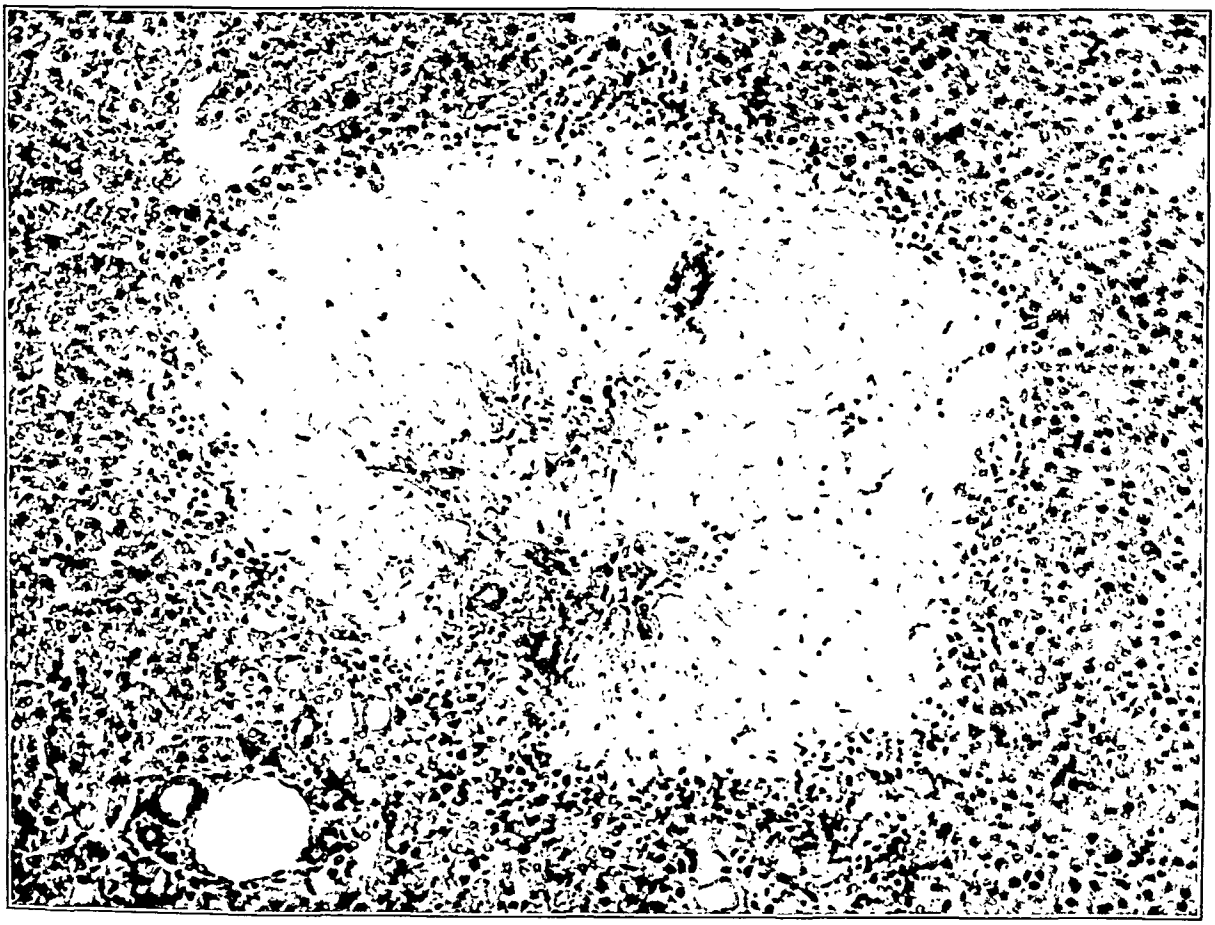
PLATE 112

FIG. 1. Photomicrograph showing a medium-sized dilated bile duct. The adjoining connective tissue is stretched and compacted. The liver cells in the surrounding lobules are elongated, compressed and atrophic. There is a slight infiltration of endothelial leucocytes into the portal area. × 150.

FIG. 2. An area of infarction involving portions of three lobules centered about a single portal area. It is sharply demarcated from the surrounding liver tissue and infiltrated at the periphery with an inflammatory exudate composed of endothelial and polymorphonuclear leucocytes. × 150.



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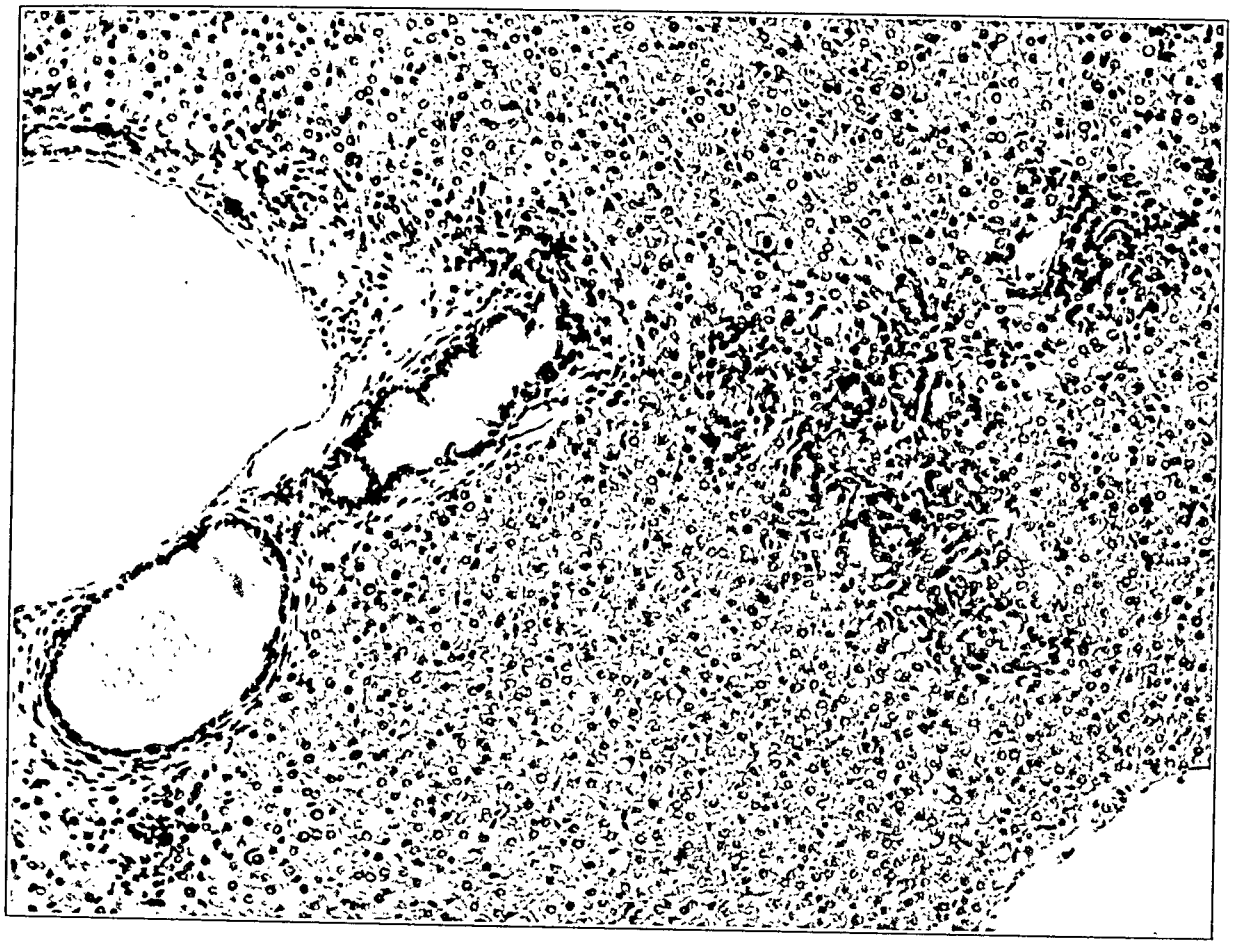


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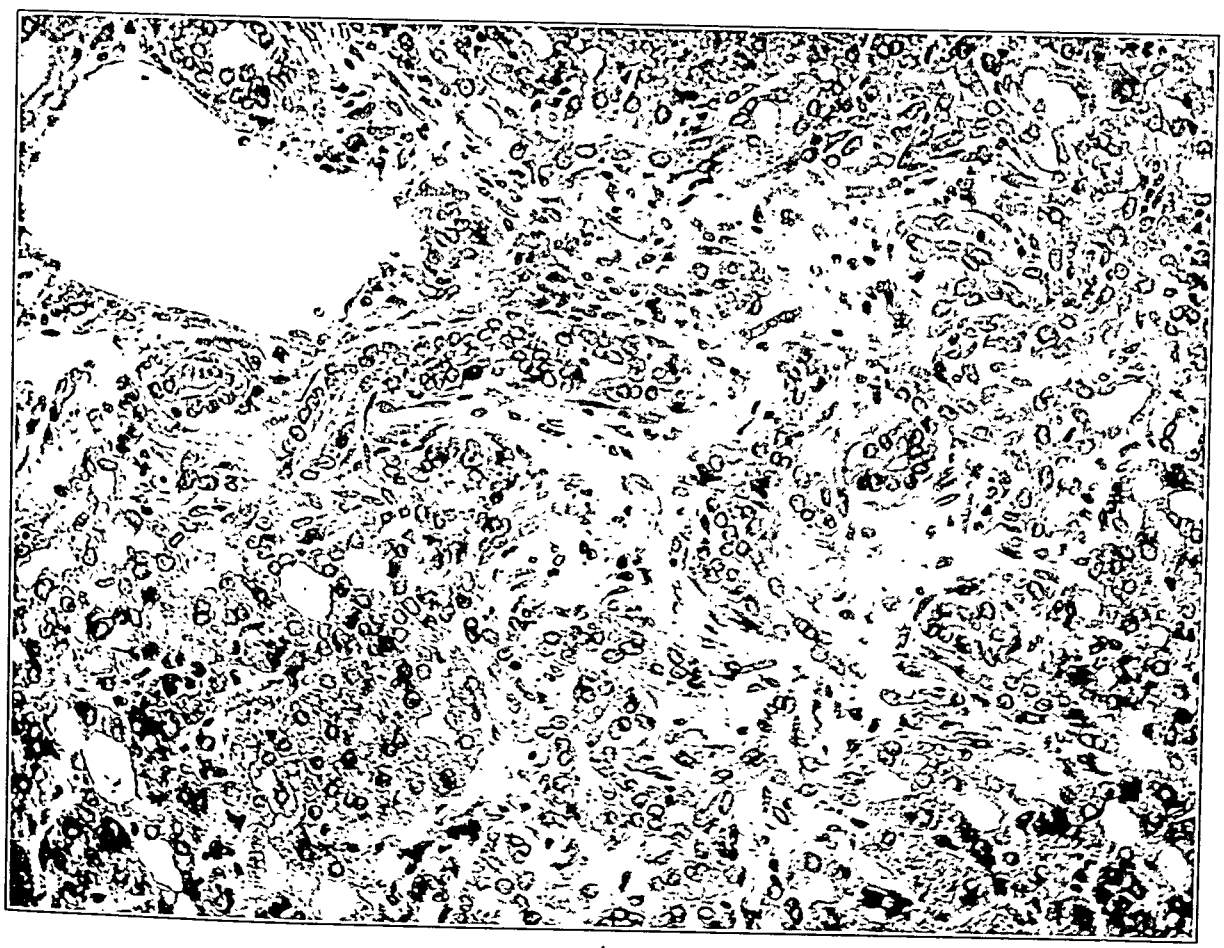
PLATE 113

FIG. 3. Photomicrograph showing distended medium-sized bile ducts and portal veins, a stretching and compression of portal connective tissue, and a slight infiltration by endothelial leucocytes. Toward the right is a smaller portal area characterized by an apparent increase in the small terminal bile ducts. × 150.

FIG. 4. A portal area in the liver of a guinea pig twelve days after ligation of the common duct showing true cirrhosis characterized by an apparent increase in the number of bile ducts and an increase in portal connective tissue. The bile capillaries in the peripheral zones of the surrounding lobules are distended into small cyst-like spaces. The liver cells surrounding the distended bile capillaries closely resemble the proliferating bile duct epithelium. Moderate infiltration of endothelial leucocytes and lymphocytes into the portal connective tissue. × 250.



3



4

MacMahon, Lawrence and Maddock Experimental Obstructive Cirrhosis

OBSTRUCTIVE CIRRHOSIS *

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(From the Pathological Laboratory of the Boston City Hospital, Boston, Mass.)

At the present time there is considerable confusion among both pathologists and clinicians in regard to the term "biliary cirrhosis." In its broader sense it is generally used to imply a form of cirrhosis confined fairly definitely to the portal areas and characterized by an increase in bile ducts and connective tissue. As regards etiology, biliary obstruction and infection are usually considered to be the important causative agents.

Several years ago Mallory [1] described an infectious type of cirrhosis resulting from bacterial infection of the smaller bile ducts. In contrast to this, the term "obstructive cirrhosis" is suggested to indicate that type which results purely from an obstruction to the outflow of bile. In either type it is clearly seen that we are dealing with a biliary form of cirrhosis in that it is the bile ducts that are primarily involved, and yet the two types are distinctly different both grossly and microscopically, and furthermore, either may be found quite independent of the other, although the two types are often combined.

The purpose of this paper is to describe briefly the gross and histological findings in obstructive cirrhosis, and to compare them with a similar form of cirrhosis which has been produced experimentally, in the absence of infection, in lower animals.

Literature

It is rather difficult to review the literature relative to this subject since little effort has been made to differentiate either the gross or histological findings of these two forms of biliary cirrhosis, namely, the obstructive and the infectious. Moreover, in infants, congenital malformations of the intrahepatic biliary system have at times not been recognized as such, and consequently have been occasionally included as forms of biliary cirrhosis of the liver.

Legg [2] in 1874 reported two cases of biliary cirrhosis, one in a

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young woman who had been jaundiced but two weeks. A gall stone was found obstructing the common duct, and the liver showed microscopically a moderate degree of interlobular cirrhosis. The second case was an elderly male with a strong alcoholic history who had been jaundiced for nine months. The autopsy revealed hydatid cysts of the liver compressing the hepatic ducts, while microscopically the liver showed considerable "portal cirrhosis."

Charcot and Gombault [3] in 1876 reported four cases of biliary cirrhosis, one of which they described as infectious cholangitis. Mangelsdorf,[4] six years later, reported a much larger series of cases with brief postmortem findings and concluded that though the histological lesions varied greatly a fibrosis of the liver was the one constant finding. Janowski,[5] Ford,[6] Eppinger,[7] Abramow and Samoilowicz,[8] and Weber [9] have likewise reported and discussed numerous cases of biliary cirrhosis, but they have not attempted to differentiate the simple obstructive from the more complicated infectious lesions.

More recently Rous and Larimore [10] in 1920 made an effort to determine the effect that bile itself played in liver lesions. From their work, which was based on experimental studies in rabbits, they concluded that no human picture resembled even remotely the lesion that could be produced in these animals as a result of ligating the common duct. Furthermore, they pointed out that attempts to utilize such experimental work in the explanation of biliary cirrhosis in man had almost been given up.

On the other hand, Snell, Greene and Rowntree [11] were able to keep dogs alive for three months after ligation of the common duct and produced what they described as an experimental counterpart of the advanced biliary cirrhosis seen clinically in association with prolonged jaundice.

Judd and Counseller [12] have quite recently studied this problem again in human cases. According to these workers, when obstruction was due to cancer, occlusion of the common duct was more rapid and more complete and a condition of hydrohepatosis developed with little or no cirrhosis. If, on the other hand, obstruction was due to a stone or a benign stricture it was usually associated with a chronic infection, and such cases showed a marked portal fibrosis and a thickening of the ducts. Here again we are left in doubt as to just how much of the lesion in the latter cases could be

ascribed to simple obstruction and how much to the complicating infectious agent.

In infants who show cirrhosis of the liver in association with congenital malformations in the extrahepatic biliary system, we are confronted not only with the problem of obstruction and infection but also with the possibility of a congenital anomaly within the liver itself.

Legg [13] in 1876 was one of the first to draw attention to a form of cirrhosis associated with congenital absence of the common duct. Thomson [14] fifteen years later described the condition of congenital obliteration of the extrahepatic bile ducts and was able to collect forty-nine cases in which the liver showed an appreciable degree of cirrhosis. This number has since been almost doubled. Rolleston and Hayne,[15] Lavenson,[16] and Howard and Wolbach [17] have reported additional cases and described cases of their own. A case reported by the last two workers has been described in greater detail and warrants further consideration. A male child, when two weeks old, developed jaundice which progressed until his death four weeks later. The liver showed a more or less advanced cirrhosis of a mixed type with both central and portal sclerosis and a good deal of interlobular connective tissue as well.

In reviewing the many different lesions in children which have been ascribed to agenesis and atresia of the extrahepatic biliary system, we find that some workers have observed changes at the periphery of the lobule, others in the center, and still others have described a more diffuse process extending throughout the lobule. In addition, the possibility of infections, toxins and congenital intrahepatic anomalies has often been overlooked in their discussions.

Lastly, Rolleston [18] in summing up the many views regarding the production of cirrhosis solely from obstruction of the common duct concluded "that biliary obstruction *per se* does not constantly cause any form of cirrhosis."

I. Studies in the Infant

Etiology: In a group of ten cases that showed varying stages of simple uncomplicated obstructive cirrhosis six were caused by congenital atresia of the common duct, two by obstruction of the hepatic ducts, one as a result of an apparent absence of hepatic ducts,

and lastly, one in which the common duct was unusually elongated, kinked and distended with thick, colorless mucus.

Holmes [19] abstracted from the literature one hundred cases showing congenital obliteration of the bile ducts which indicates that this anomaly is not an extremely rare condition. Moreover, he pointed out that the obstruction may be single or multiple, may involve any portion of the ducts, and lastly, in at least sixteen of the cases surgical relief would have been possible.

Congenital absence of the common duct is a very much more uncommon finding. Kirmisson and Hébert [20] reported a case in which there was no trace of either hepatic, cystic or common duct, and were able to collect from the literature two cases similar to their own.

A few other examples of obstruction of the common duct which have been reported in infants were caused by the presence of large lymph nodes which pressed on the common or hepatic ducts.

In regard to the nature of atresia of portions of the extrahepatic biliary system, the most commonly accepted viewpoint, and one described by Beneke,[21] is that it is primarily a developmental anomaly. However, there are others who regard this condition as the healed stage of an acute cholangitis that occurred during fetal life, induced by maternal toxins and infections. Futhermore, there are those who believe that the cirrhotic changes in the liver are the result of an inflammatory reaction in that organ during fetal life, and that atresia of the ducts represents merely an extension of the process down into the larger bile passages.

We believe that the congenital atresia resembling congenital agenesia of the extrahepatic biliary system is primarily a developmental anomaly, that the cirrhotic changes in the liver are entirely secondary, and furthermore that the picture of uncomplicated obstructive cirrhosis is entirely different from the intrahepatic developmental anomalies involving either the bile ducts or the mesenchyma.

Gross Appearance of the Liver: In the early stages the liver, apart from a marked greenish discoloration, may appear quite normal. Even in the later stages the cirrhotic changes are relatively less conspicuous than in other forms of cirrhosis. The surface is uniformly covered with very fine granulations resembling fine Morocco leather. The liver is not increased in size, it is firm and cuts with increased resistance. In cases of very long standing the portal areas appear unusually accentuated on the freshly cut surface.

Microscopic Appearance of the Liver: The earliest cases in which we were able to see any changes other than the picture of simple bile stasis were in infants who had lived at least three weeks. Before this one finds bile within the liver cells, distending the bile capillaries, especially of the central zones, bile in the terminal and collecting bile ducts, and at times in the perisinusoidal spaces. The liver cells may be rather swollen, may vary somewhat in size and contain two or more nuclei, but mitoses are infrequent. The Kupffer cells are unusually conspicuous, apparently increased in size and number, and may contain droplets of inspissated bile. But with all this, there is no appreciable change within the portal areas except a slight infiltration by endothelial leucocytes, a few of which may contain blood pigment and bile.

The beginning of the cirrhotic process is characterized by an apparent increase in the terminal bile ducts. The bile duct epithelium proliferates as shown by the presence of mitoses and the ducts become lengthened and tortuous, but no new bile ducts are formed. This process is going on in every portal area throughout the liver so that one finds this change bordering the larger portal areas as well as in the smaller. Moreover, since the effects of the obstruction are equally distributed throughout the terminal bile ducts, any changes that they may undergo are carried out in a uniform manner throughout the liver. At the periphery of the lobule the bile capillaries are frequently so distended that they compress the bordering liver cells, and in following the lumina of the bile capillaries into the terminal bile ducts it is practically impossible to determine where liver cells stop and bile duct epithelium begins.

Accompanying this elongation of terminal bile ducts there is a gradual proliferation of fibroblasts in the portal areas so that more and more connective tissue is formed in the portal areas as the process progresses.

The whole process is so gradual that the circulation has an opportunity to adjust itself to these changes so that infarcts, which are invariably found in animals in which the common duct has been suddenly ligated, are extremely uncommon.

In the later stages, the portal areas are uniformly increased in size throughout the liver, partly as a result of the apparent increase in bile ducts due to elongation of them, and partly owing to an absolute increase in the amount of connective tissue. Along with this

change, the lobules have diminished somewhat in size, although even in those cases which had lasted three to four months we found neither central sclerosis nor an appreciable increase in the interlobular connective tissue.

Clinical and Pathological Aspects: One of the most variable findings in an infant's liver is the amount of bile in liver cells and bile capillaries. Kölliker [22] found that the secretion of bile begins as early as the third month of embryonic life; however, it is probable that this would be delayed considerably and diminished in cases of atresia. Furthermore, the degree of blood destruction varies considerably in this group of infants, a condition which, as pointed out by Rous and Drury,[23] largely regulates the quantity of pigment in the bile.

In our small series of ten cases, jaundice had been present since birth; however, there are innumerable cases reported in which jaundice did not appear for days or weeks later. According to Porter,[24] "jaundice is the predominant symptom and while it may be present at birth, its appearance is sometimes delayed until toward the end of the first month." In cases of complete congenital atresia, this delayed appearance of jaundice is difficult to understand.

Various gross changes have been reported in the liver and other organs. Many authors have described both liver and spleen as being greatly enlarged, while others have found fibrosis in the pancreas. In our small series the liver was of normal size; no constant changes were seen in the pancreas, nor was there any evidence of portal obstruction as characterized by an enlarged spleen, varices or ascites.

A clinical summary of our cases shows that jaundice, hemorrhages, pigmentation of the urine and clay-colored stools were the outstanding features. Nutrition in most cases was well maintained for some time in spite of the severity of the lesion, but later the health inevitably gave way and the child rapidly lost weight and strength. The longest surviving case that we encountered lived for four and a half months; however, reports of six to eight months' survivals are not uncommon in the literature, while two cases are said to have lived nine months, one until the eleventh, and one reported by Porter, as long as the fourteenth.

II. Studies in the Adult

Etiology: Of twenty cases of obstructive cirrhosis in adults that came to autopsy, eight resulted from tumors about the head of the pancreas and ampulla of Vater, eight from stones in the common duct, and four from benign strictures. Of this series only five of the cases were females and it is interesting to note that in each of these cases the obstruction was due to stones. The average age of the males was about 55 years and of the females about 60, although in both groups there was a wide variation.

Gross Appearance of the Liver: The liver was normal in size and contour, and the surface was quite smooth except in those cases of long standing when it was uniformly and very finely granular. The consistence also varied somewhat, but only in the later stages had it appreciably increased, and then it was never so marked as in long standing cases of alcoholic, pigment, toxic or infectious cirrhosis. In all of the cases except one the whole organ was deeply bile-stained; the one case which was an exception had been caused by a stone that was removed a month or two before the patient came to autopsy.

Microscopic Appearance of the Liver: This differs slightly in adults and children, and in the former, seldom if ever, becomes such a conspicuous feature as one finds in the long standing cases in infants. Furthermore, in elderly patients, where obstruction has existed for many weeks, atrophy and degeneration of the parenchyma gradually become a more prominent part of the picture. In addition to these minor variations that are associated with the age of the individual, one occasionally finds a combination of two or more types of cirrhosis, such as obstructive and infectious, or obstructive with an alcoholic or pigment cirrhosis, and in such cases either the gross or histological picture may become extremely complicated.

In the early period of biliary obstruction the pathology is simply that of bile stasis, as was the case in both infants and animals, but in adults the picture is more clearly defined, and one has a better opportunity to study the changes in the bile, in the liver cells, in bile ducts and in the connective tissue stroma.

Bürker [25] has divided the lobule of the liver into two parts, namely a central portion for secreting and a peripheral portion for absorbing, and such an hypothesis would seem to be substantiated, at

least in part, in cases of bile stasis. A few days after biliary obstruction, while bile is still being actively secreted, the liver cells in the central zones show more or less regressive changes. The cells are irregular in size and shape, the cytoplasm may stain quite intensely or appear unusually pale, in a few cells it may scarcely stain at all, while here and there one sees a necrotic bile-stained cell that is being invaded by endothelial leucocytes. The nuclei also vary in size, contour and in the arrangement of the chromatin; and liver cells with three, four and five nuclei are not at all uncommon, while occasionally, though rarely, mitoses are also found. The bile that lies in the cytoplasm of the liver cells sometimes appears as yellowish and green granules; at other times as large and small droplets. It may be clumped in a small focus bordering the nucleus, it may form a complete halo about the nucleus, and lastly it is sometimes diffusely scattered throughout the cell. Bile that is homogeneous and orange to greenish, distends the bile capillaries but can seldom be traced into the lateral canaliculi between the liver cells. The Kupffer cells, especially those in the central zones, are increased in number, swollen and contain bile in the form of granules and droplets. The inspissated masses of bile in the dilated bile capillaries appear cylindrical, irregular and branching and vary from orange to dark green. They are often extruded and lie between the liver cells and the walls of the sinusoids. Here they are phagocytosed by endothelial leucocytes which in consequence may become greatly enlarged and occasionally multinucleated. The leucocytes sometimes accumuate in considerable numbers and necessarily compress the sinusoids. The result is that the picture in the centers of the lobules is often complicated because of the difficulty of distinguishing the endothelial cells lining the sinusoids from the endothelial leucocytes attracted out of the circulation. Endothelial leucocytes containing granules and droplets of bile are often present also in the lymphatics and stroma of the periportal tissue.

A little later, though the liver cells may still show retrograde changes, and be swollen and vacuolated, they contain less bile, which usually lies clumped about the nucleus. Here and there one finds bile in both liver cells and Kupffer cells throughout the lobule, but this is not common and in such cases the quantity of bile in the periphery is much less than in the centers of the lobules. At this stage the number of bile-stained endothelial leucocytes may be in-

creased in the portal areas, but usually there is no apparent change in either the bile ducts or portal connective tissue.

The earliest structural changes to become manifest were found in two cases in which the common duct had been completely blocked by large biliary calculi for a period of three weeks. In all the portal areas the terminal bile ducts were unusually prominent, they were filled with bile and showed an apparent increase in number, while associated with this change was a slight increase in portal connective tissue.

We have followed the progressive histological changes in both bile ducts and connective tissue in cases in which complete obstruction has existed for as long as eighteen months, in a second group of cases in which obstruction has been incomplete for an equal length of time, and lastly in a group in which obstruction has been intermittent over a period of three to five years.

The changes in all cases have been much the same, the difference lies only in degree. The bile duct epithelium of both collecting and terminal ducts proliferates, and there is a progressive elongation of the periportal bile ducts which become tortuous and much more conspicuous. The portal connective tissue is increased and in it one finds an infiltration by endothelial leucocytes, lymphocytes, scattered eosinophiles and basophiles. If the obstructing agent, such as a stone, has been removed some time before death, bile stasis will probably be absent, and the elongated bile ducts in the portal areas are much less conspicuous.

In brief, then, the picture that one usually finds in an uncomplicated case of biliary obstruction of several weeks duration is characterized by an increase in size of the portal areas due to an elongation of the terminal bile ducts, a proliferation of connective tissue, and an infiltration by both endothelial leucocytes and lymphocytes. The liver cells in the central zones show mildly regressive changes and inspissated bile fills the bile capillaries.

However, other changes occur with sufficient regularity to warrant further consideration. In six of the twenty livers which have been included in this series there were infarcts which varied in number, in size and in the stage of resolution. They usually involved only a portion of a lobule and in all cases radiated out in a fan-like manner from the portal areas. Furthermore, they appeared in cases where the obstruction had occurred quite suddenly and had been

quite complete. These infarcts, when first formed, resemble those that are constantly found in the livers of lower animals following experimental common duct ligation;[26] there was little extravasated blood, slight peripheral reaction and no discoloration with bile. Later there is phagocytosis of this necrotic tissue by encroaching endothelial leucocytes and finally even these cells disappear leaving a rather loose structureless area of connective tissue.

Another lesion that sometimes may be quite confusing in long standing cases is the cellular reaction to old inspissated bile that lies free in the portal connective tissue. Apparently as the result of pressure, the epithelium lining the small ducts may become greatly stretched and finally disappear leaving a large tubular mass of inspissated bile surrounded by connective tissue. The bile acts simply as a foreign body, such as agar, and is soon attacked by endothelial leucocytes and foreign body giant cells, while still later fibroblasts proliferate about it and traverse it giving it the appearance of an old, partially organized thrombus.

In two cases in which obstruction had been present about six weeks there was a small irregular accumulation of bile in the connective tissue of two or three of the medium-sized portal areas suggesting that there had been a rupture of one of the smaller bile ducts. There was a zone about this, infiltrated with a few polymorphonuclear and endothelial leucocytes, but there was no necrosis of the liver cells in the surrounding lobule.

A change in the portal vessels was a very uncommon finding and occurred in only one of our cases. As a result of a benign stricture of inflammatory origin about the lower end of the common duct there had been complete obstruction to the outflow of bile for more than a year. In this case there was a very marked subintimal proliferation of connective tissue in the medium-sized branches of the portal vein, which considerably narrowed the lumen. Moreover, this was the only uncomplicated case of obstructive cirrhosis showing ascites. It is well recognized that as a result of bile stasis the pressure within the portal circulation is increased, and presumably if other cases had progressed for longer periods of time a secondary sclerosis of the vessels would not have been such an unusual finding.

In two cases of long standing biliary obstruction, both in elderly people, there were small scattered foci, most numerous in the central zones, in which the liver cells had disappeared leaving the trabecu-

lar spaces filled with fatty and pigmented endothelial leucocytes. A similar observation was described by Ogata [27] who attributed it to the presence of retained bile within the liver cells of the secreting portion of the lobule. However, though degenerative changes as well as occasional necrotic cells were frequently seen within the central zones, larger areas from which the liver cells had entirely disappeared were very much rarer and should probably be explained as due not alone to the action of the bile but also to the impairment of the liver circulation and lack of regenerative activity on the part of the remaining cells.

In reviewing these more unusual changes that are sometimes found, it is apparent that a well marked case of obstructive cirrhosis in an adult may show not only a dilatation and elongation of bile ducts and an increase in the portal connective tissue, a variable degree of bile stasis and retrograde changes in the centers of the lobules, but also infarcts, organizing bile thrombi, evidence of ruptured bile ducts, sclerosed vessels and small irregular foci in which the liver cells have disappeared.

Clinical and Pathological Aspects: Jaundice that was intense, enduring and unassociated with signs of sepsis, was present in all cases. However, the duration of life after the onset of jaundice varied greatly not only in cases due to different causes but also in different cases resulting from a similar cause; thus in cases of obstruction due to tumors and gall stones it ranged from three weeks to six months averaging in all about two months, while in three of the cases associated with gall stones there was in addition a history of intermittent jaundice for two, three and five years. In cases due to stricture, of which we have included but four, jaundice was present for two, three, eight and eighteen months respectively.

The patients generally had fairly good health at first, but toward the end of life, gastro-intestinal disturbances, loss of weight and strength were almost constant findings.

The liver was normal in size, and only in long standing cases showed appreciable gross evidence of cirrhosis. Furthermore, there were no outspoken signs of portal embarrassment such as enlargement of the spleen, ascites, hematemesis or varices, except in one patient who lived eighteen months after the onset of jaundice. These are rather important points because they assist in the differential diagnosis of this form of cirrhosis.

COMMENT

Comparatively little attempt has been made in the diagnosis of the various forms of cirrhosis to differentiate as a distinct and characteristic type that form which is found in prolonged and uncomplicated obstruction to the common bile duct. One reason for this has probably been its relative lack of importance, but another and more obvious reason is that it has been loosely crowded into that rather heterogenous group labeled "biliary cirrhosis," or included among those in which there has been a terminal bile duct infection.

It would appear that obstructive cirrhosis in childhood is not materially different from the lesion one finds in the adult, and it is precisely the same in principle as one finds in the lower animals subjected to ligation of the common duct.

In the study of autopsy and surgical material from human cases both in children and adults we have had an opportunity to follow each stage in the development of this form of cirrhosis. In the lower animals, such as the guinea pig, changes occur with great rapidity, and it would also appear that in the infant, cirrhosis develops more quickly than in the adult, at least it becomes very much more conspicuous in a similar period of time. The early stage in all cases is characterized by a dilatation of the ducts, with bile stasis within the lobule. This is followed by an elongation of the terminal ducts associated with an appreciable increase in portal connective tissue, commonly accompanied with a moderate cellular infiltration into the portal areas and the appearance of regressive changes in the liver cells in the central zones of the lobule.

With biliary obstruction there is an impairment in the hepatic circulation, although only very rarely are there visible signs of interference with the portal circulation.

SUMMARY

1. This paper is based on the study of thirty cases of uncomplicated obstructive cirrhosis, ten in infants and twenty in adults averaging about sixty years of age.

2. The common cause in infancy is a congenital malformation of the bile duct, whereas in the adult it results from tumors, concretions and inflammatory strictures.

3. The gross and microscopic findings of obstructive cirrhosis are described, and in addition, reference is made to a few of its more important clinical features.

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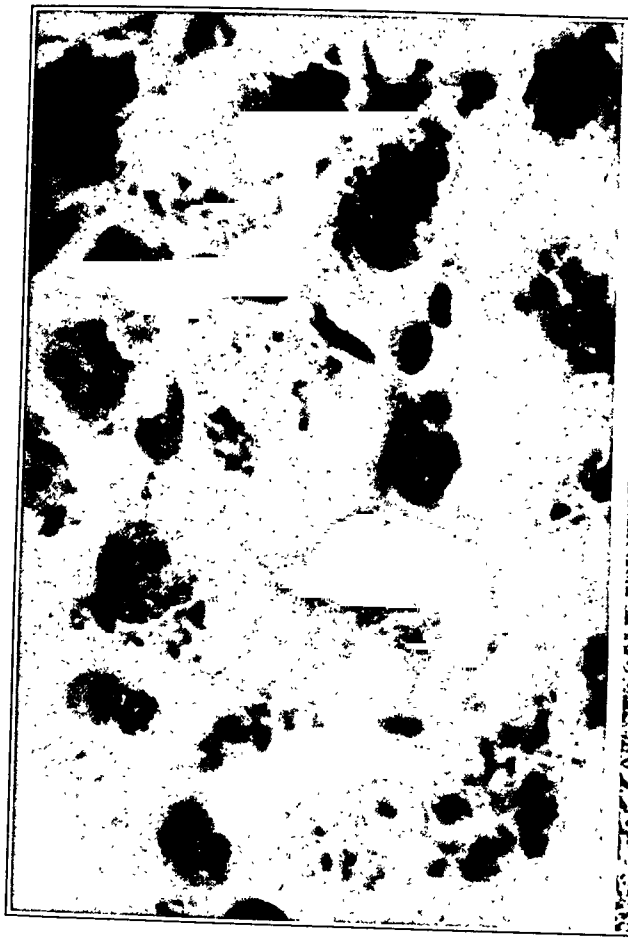
DESCRIPTION OF PLATES

PLATE 114

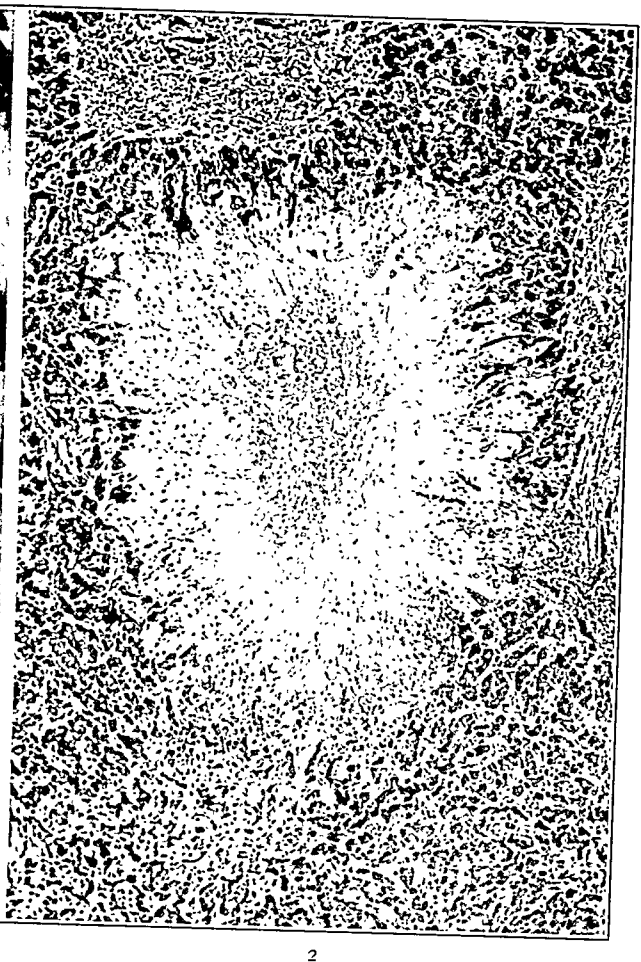
FIG. 1. OS 29.18. Section of a bit of liver tissue removed from a man 40 years of age who was operated on for acute obstruction of the common bile duct. Recovery was complete. The liver cells are enlarged and contain numerous granules and droplets of bile. In a dilated bile duct is a branching mass of inspissated bile. × 1000.

FIG. 2. 23.44. Obstructive cirrhosis secondary to cicatricial contraction around the ampulla of Vater in a man 75 years of age. Jaundice for two months. The photomicrograph shows an infarct with beginning organization at the periphery. × 80.

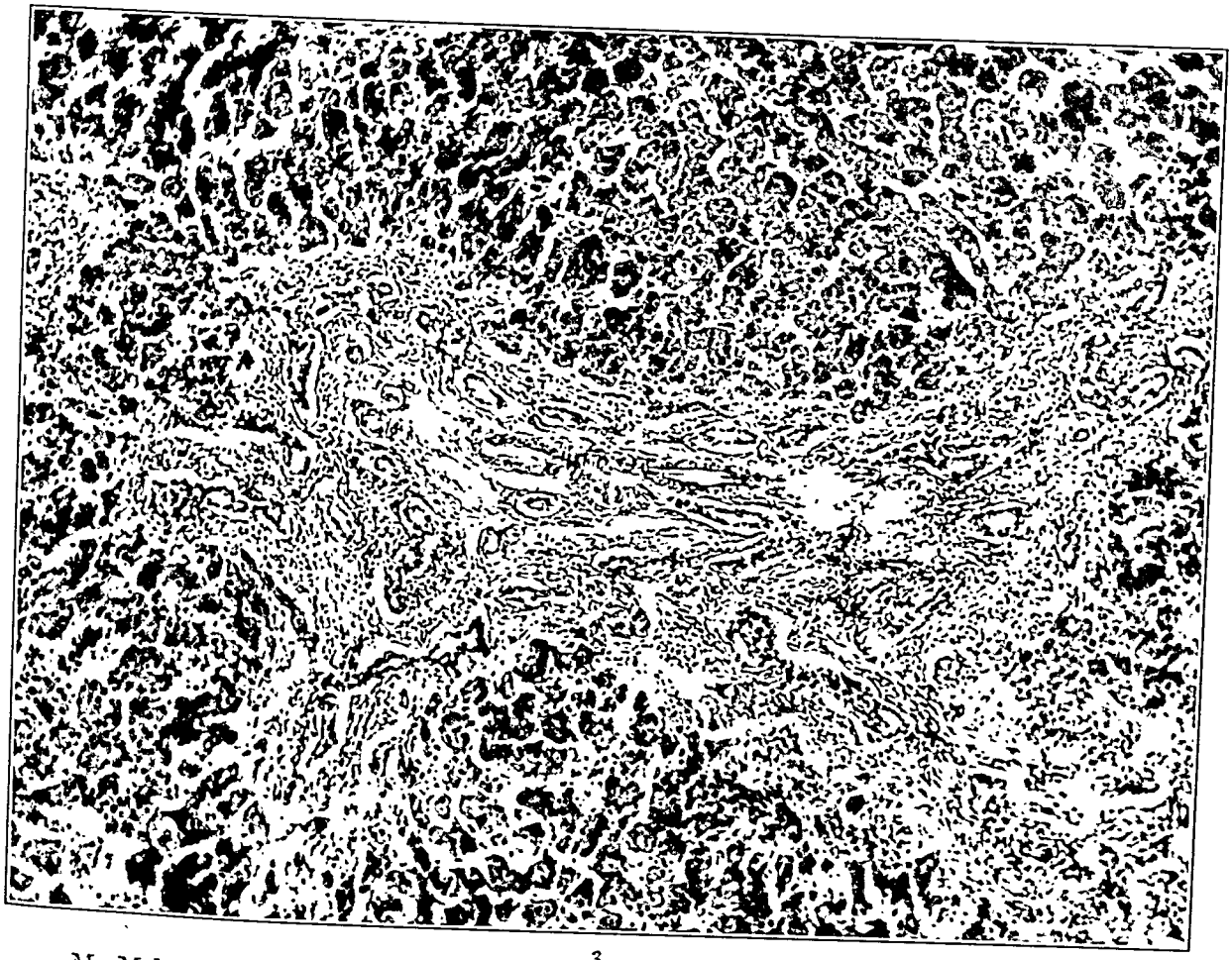
FIG. 3. M 1074. Female infant 28 days old. Congenital obstructive cirrhosis. A portal area showing increase of connective tissue and a number of bile ducts. × 100.



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2



3

MacMahon and Mallory

Obstructive Cirrhosis

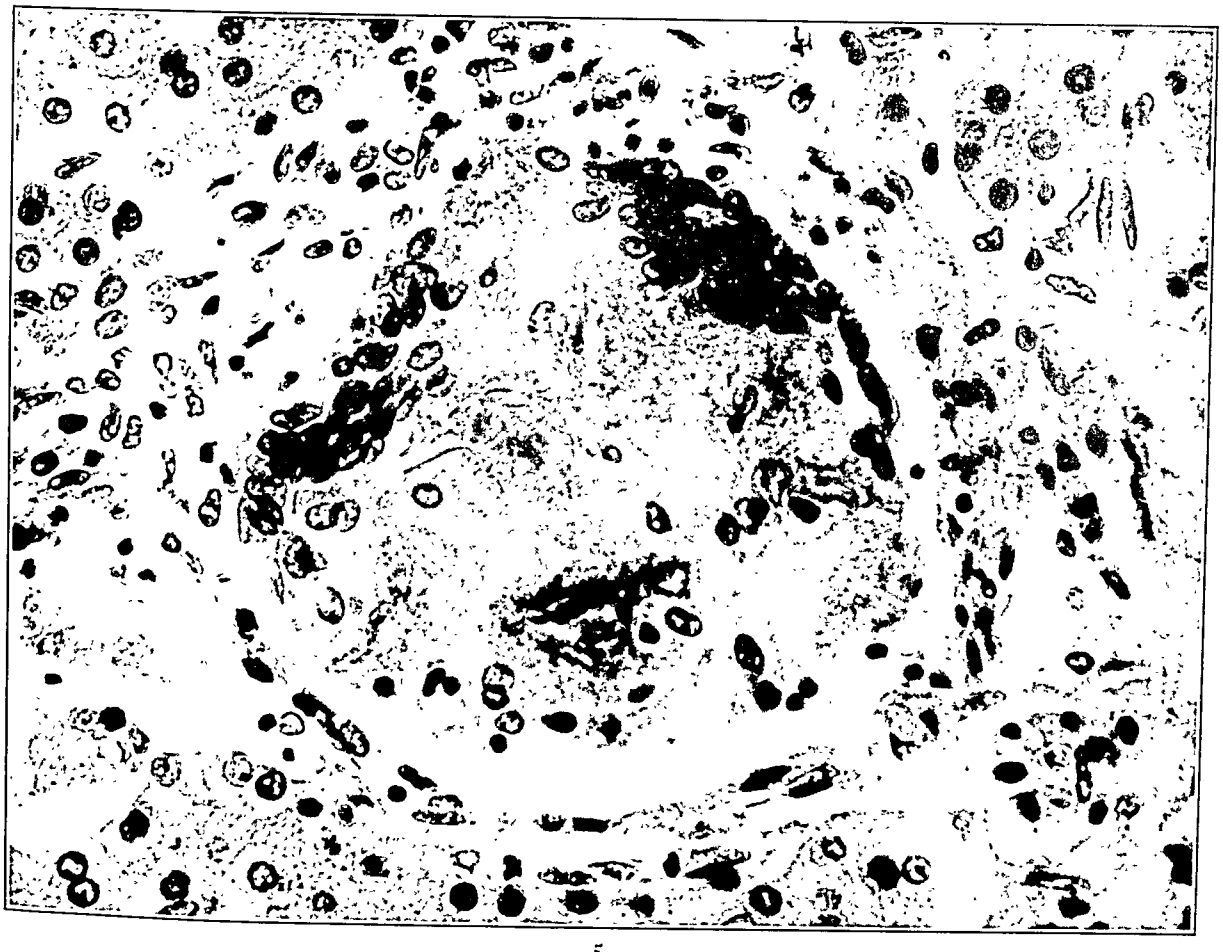
Plate 115

Fig. 4. 22.298. Obstructive cirrhosis of considerable duration in a man 54 years of age. Two masses of old inspissated bile in bile ducts are undergoing organization. The periportal connective tissue is greatly increased in amount and has numerous bile ducts included in it. × 100.

Fig. 5. M. 1074. From same liver as shown in Fig. 3. A mass of inspissated bile in a bile duct surrounded and infiltrated by endothelial leucocytes and foreign body giant cells. × 500.



4



5

MacMahon and Mallory

Obstructive Cirrhosis

STUDY OF A MENINGIOMA IN SUPRAVITAL PREPARATIONS, TISSUE CULTURE AND PARAFFIN SECTIONS *

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Tissue cultures of intracranial tumors removed at operation in this clinic have been successful for certain of the gliomas, such as the glioblastoma multiforme and the medulloblastomas. Outgrowths of cells have been observed in some of the thirty-one meningiomas cultured during the past two years but as Kredel [1, 2] has previously stated, the growing cells seemed to be of the mononuclear macrophage series and not tumor cells. From their phagocytic properties and growth pattern, the cells were considered to be clasmatocytes which were seen abundantly in the supravital preparations of the meningiomas.

Recently a rapidly growing meningioma was removed at operation and portions of the tumor were studied in immediate supravital preparations, in tissue cultures of the same bits of tissue, and in stained paraffin sections. The cells growing out in culture in a pattern like that of cells of mesodermal origin, closely resembled the tumor cells seen in the supravital preparations and in the paraffin sections. Phagocytic cells were not observed among the growing cells. The growing cells did not appear to be of the fibroblastic type and in the stained sections of the fixed tissue the cells had no resemblance to fibroblasts.

This single instance of a successful tissue culture of a meningioma is presented because it shows by the growth pattern the mesodermal origin of the cells of this tumor, that the tumor cells had no demonstrable phagocytic powers, and that the real phagocytic elements were clasmatocytes which are so commonly found in human and animal tumors.

Parasagittal Meningioma Localized by Ventriculography. Extirpation. Recovery

Clinical History: Case P.B.B.H. Surgical No. 33356. On February 23, 1929, William M. was referred by Dr. Howard F. Root of Boston, with a diagnosis of tumor of the brain. For three years before admission the patient was known

* Received for publication July 31, 1929.

to have had diabetes mellitus in a mild form and was treated accordingly. Because of an exacerbation of this illness he was admitted to the Deaconess Hospital for observation. While there he complained of diminution in vision, suboccipital headaches and unsteadiness in gait which had been present for the preceding two months. The ophthalmological examination showed a bilateral choking of the optic discs and the patient was transferred to this hospital.

The admission examination showed a papilledema of 1.5 diopters on the right and 2.5 diopters on the left. The visual fields were constricted to tubular vision in each eye. There was suboccipital tenderness and stiffness of the neck. Signs of a subtentorial lesion were found in the hypotonia of the upper extremities and adiadokocinesia of the left side. The roentgen-ray examinations of the skull and chest were considered normal.

Course of Illness: On the day after admission the previously demonstrated signs of a cerebellar lesion were less distinct and in view of the increasing papilledema and absence of localizing symptoms a right subtemporal decompression was made on March 12, 1929. This procedure gave but temporary relief and as the intracranial pressure increased ventriculography was made nine days later. From the deformity of the ventricles a right frontal tumor was suggested and a right osteoplastic intracranial exploration was made and a large vascular parasagittal meningioma was removed by Dr. Cushing. The operation was a long and difficult one as was the postoperative course.

At present, June 26, 1929, the patient reports as perfectly normal and at work.

The observations on the supravital preparations, tissue cultures and paraffin sections are given in the order in which they are routinely made for the intracranial tumors removed in this clinic.

Supravital Studies: Preparations of the fresh tumor with neutral red dye solution were made immediately at operation. Examination showed a densely cellular tissue. The cells were connected one to another, forming narrow columns which were typical of the general architecture of the tumor (Fig. 1*a*). The cell bodies were oval or polyhedral with a delicately granular cytoplasm nicely defined, except in areas so crowded with cells that only the nuclei could be clearly distinguished. There was no evidence of cellular division. The nuclei were oval or round, containing fine chromatin particles and one or more nucleoli (Fig. 1*b*). A few strands of collagen were present (Fig. 1*d*).

Most conspicuous in the preparation were the extraordinary numbers of clasmatocytes scattered among the tumor cells or arranged in clumps. During the period of observation they became packed with neutral red dye inclusions which varied from very fine granules to vacuoles huge in size (Fig. 1*c*).

Tissue Culture Studies: Numerous tissue culture preparations were made by the hanging-drop method. Tissue adjacent to that

used for supravital preparations was used. In each of thirty separate cultures there was seen extension of cells from the explant into the culture media. The cells were all of the same type and grew out in a reticular pattern as do cells of mesodermal origin (Figs. 2 and 3). In eight of the cultures the growth was profuse, the cells extending to the periphery of the growing space in four days.

The first evidence of growth was seen five hours after the cultures were planted when single cells, with oval bodies and long processes connected to the explant, jutted out into the media. The cells grew out evenly and were always connected with each other by processes extending from one cell body to another. This gave a reticular pattern to the growth. No mitoses or direct cell divisions were seen. The cell bodies varied in shape and were usually oval or triangular. Changes in the shape of the cell bodies were seen only at the periphery of the growth. No motile cells and no cells resembling clasmatocytes were seen among the growing cells although such cells were recognized in the explants.

The phagocytic powers of the cells of the culture were tested by adding red blood corpuscles of the patient and of other patients, as well as carmine particles to the culture media. There was no evidence of phagocytosis among the cells although the materials remained in the culture media for four days in several preparations.

The cultures were fixed in 1 per cent formalin and in formalin ammonium bromide and stained successfully with hematoxylin and eosin and unsuccessfully by Cajal's gold chloride sublimate method.

Pathological Studies: The tumor weighed about 115 gm. The overlying dura and bone were roughened, thickened and involved by the encapsulated tumor which resembled other meningiomas seen in this clinic.

Microscopic Studies: The paraffin sections were stained with eosin-methylene blue, phosphotungstic acid hematoxylin and by Perdrau's method for connective tissue. The tumor is densely cellular, moderately vascular and sharply marked off from the small portions of compressed cerebral tissue attached to the specimen (Fig. 4). The cells are closely packed together and are often arranged in short or long strands or cords (Fig. 5). There is neither whorl formation, calcification nor evidence of degeneration in the material studied.

The cells are similar and are oval or polyhedral with oval or

rounded nuclei and a moderate amount of cytoplasm. Each nucleus contains one or several nucleoli and occasionally mitoses are seen. The individual cells show no evidence of laying down specific substances. The small amount of collagen present is usually near blood vessels. The stroma is scanty and phagocytic cells are not recognized. The cell type and arrangement are similar to those seen in sections of several recurrent and atypical meningiomas.

To summarize, the cells of this tumor as seen in supravital preparations, in growing tissue cultures, and in paraffin sections appeared to be of the same type. Clasmatocytes were seen in the supravital preparations and in the explants, but were not recognized in the paraffin sections.

It is our opinion that the cells which grew out in culture in a reticular pattern, typical of cells of mesodermal origin, were tumor cells.

Comment

It is known that in the tissue culture of animal tumors, either sarcoma or carcinoma, different types of cells grow out. Certain of these cells were recognized by Lambert and Hanes [3] as stroma cells which were distinct from the tumor cells in their pattern of growth. They also found that the stroma cells were actively phagocytic and ingested carmine particles.

Lewis and Gey [4] described as clasmatocytes the cells called stroma cells by Lambert and Hanes, and found that they were present both in the supravital preparations and the tissue cultures of mouse sarcoma.

That there has been some question as to whether scavengers, macrophages or clasmatocytes so frequently found in animal tumors are malignant cells or not is evidenced by the work of Fell and Andrews.[5] From their studies made by the hanging-drop method, these investigators were unable to tell which of the two cell types found in cultures of Jensen's rat sarcoma was the malignant cell.

Carrell and Ebeling [6, 7, 8] answered this problem, for by their most refined tissue culture methods they were able to isolate the fibroblastic and the macrophage cells and to prove that the former cell was the real tumor cell of the Jensen's rat sarcoma. They also found that pure cultures of animal tumor cells may be grown *in vitro* but *in vivo* the tumor cells need the macrophages or clasmatocytes for their growth. Furthermore they showed that in tissue cultures pure

cultures of animal tumor cells attracted clasmatocytes. Finally it was their opinion that clasmatocytes stimulated the proliferation of fibroblasts and tumor cells in tissue culture, probably playing a rôle in the nutrition of growing cells by aiding in the furnishing of food to these cells.

In the many immediate supravital preparations made from portions of intracranial tumors removed by Dr. Cushing, clasmatocytes were commonly observed especially in the smears from the rapidly growing gliomas and meningiomas. The clasmatocytes may be considered as a normal constituent of these tumors *in vivo*, as they are so considered in certain animal tumors.

In the reports of his investigations of the tissue cultures of intracranial tumors removed in this clinic, Kredel [2] stated that in six of nine meningiomas there were outgrowths of cells which seemed to be of the mononuclear macrophage series which were proved to be actively phagocytic and most probably clasmatocytes. He further stated that the cells growing out from the explants of meningiomas did not grow in the reticular pattern as do fibroblasts nor did they behave as do fibroblasts in the presence of arsenious trioxide.

Tissue cultures have been made of twenty-two meningiomas presenting a histological structure similar to that of the other meningiomas cultured. In twenty-one of these tumors there was an abundance of collagen, psammoma bodies and cellular whorls; certainly not the type of a tumor from which a growth of tumor cells in tissue culture could be expected. In but one instance, that of the case herein reported, has there been an outgrowth of cells which could be considered as tumor cells, although cells regarded as clasmatocytes were observed.

As stated, the tumor of the case presented was considered to be a meningioma from its gross appearance and from the histological study of both the paraffin sections and the supravital preparations. In the tissue cultures there was an abundant growth of similar cells always in the form of a reticular pattern. These cells resembled the cells of the tumor as seen in the supravital preparations. They were found not to have phagocytic properties. They were considered to be the tumor cells of the meningioma, and of mesodermal origin as their pattern of growth was like that of cells of mesodermal origin. They did not resemble cells which are fibroblastic in origin, for no substances were seen to be laid down by the cells.

Summary and Conclusions

1. The tumor cells of a meningioma were found to be similar in type and arrangement in supravital preparations, tissue cultures and paraffin sections.

2. Two types of cells were recognized in the supravital preparations, one the tumor cell and the other a macrophage or clasmatocyte. The latter type of cell seems to be essential for the continued growth of animal tumor cells *in vitro* and probably *in vivo*.

3. In tissue cultures the tumor cells of the meningioma grew in a reticular pattern as do cells of mesodermal origin and were not found to have phagocytic powers. It would seem that the phagocytic cells seen in meningiomas are clasmatocytes and not tumor cells.

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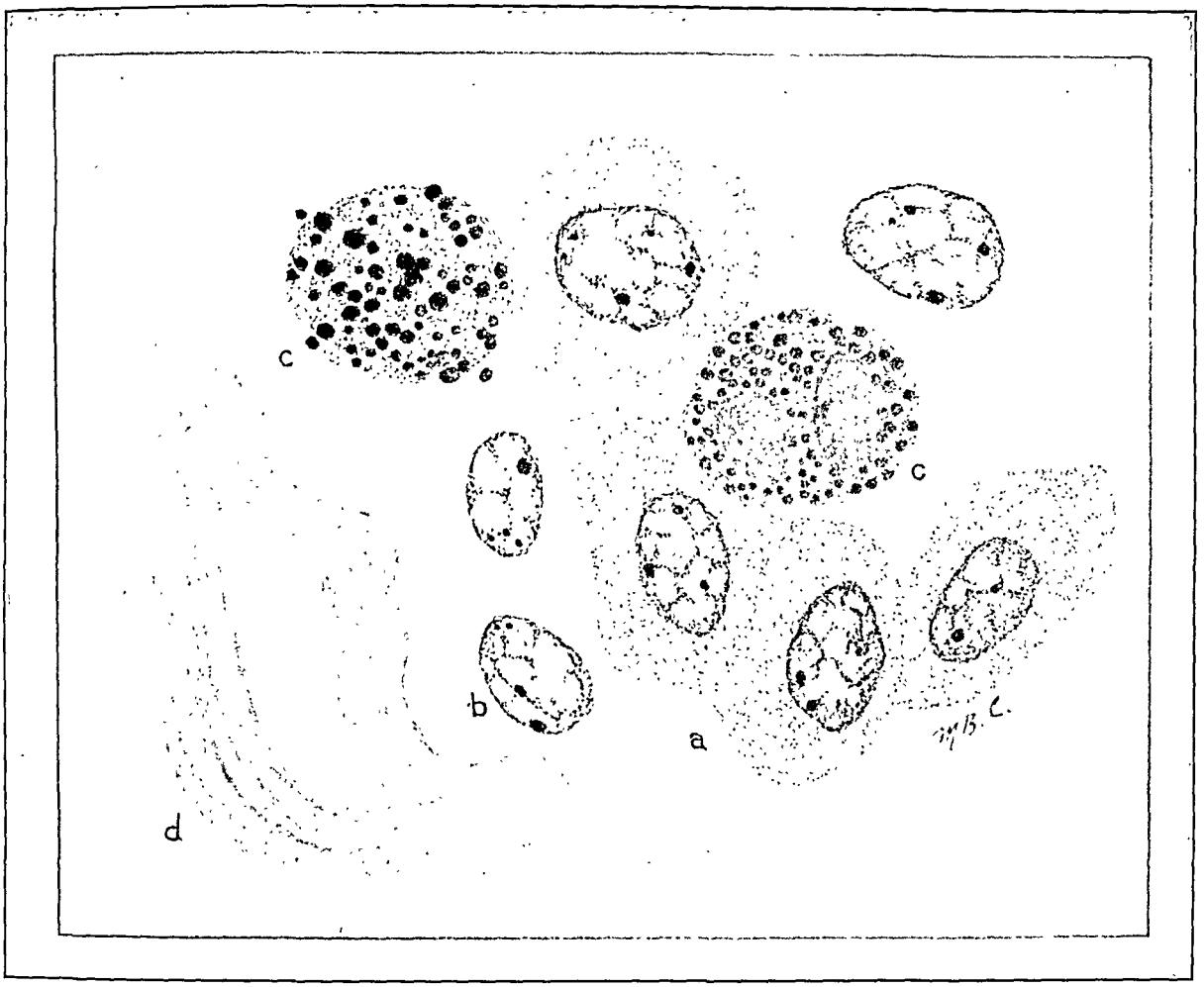
DESCRIPTION OF PLATES

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Fig. 1. Drawing from original sketch from supravital preparation showing: (*a*) a typical column of tumor cells; (*b*) the vesicular nuclei; (*c*) clasmatocytes filled with neutral red dye inclusions; (*d*) strands of collagen.

Fig. 2. Showing the reticular outgrowth of cells from the explant (*A*) typical of mesodermal tissue and the grouping of some cells in small cords as seen in all of the cultures. The fixation in Zenker's fluid and the subsequent staining in hematoxylin and eosin have necessarily altered the cells. × 300.

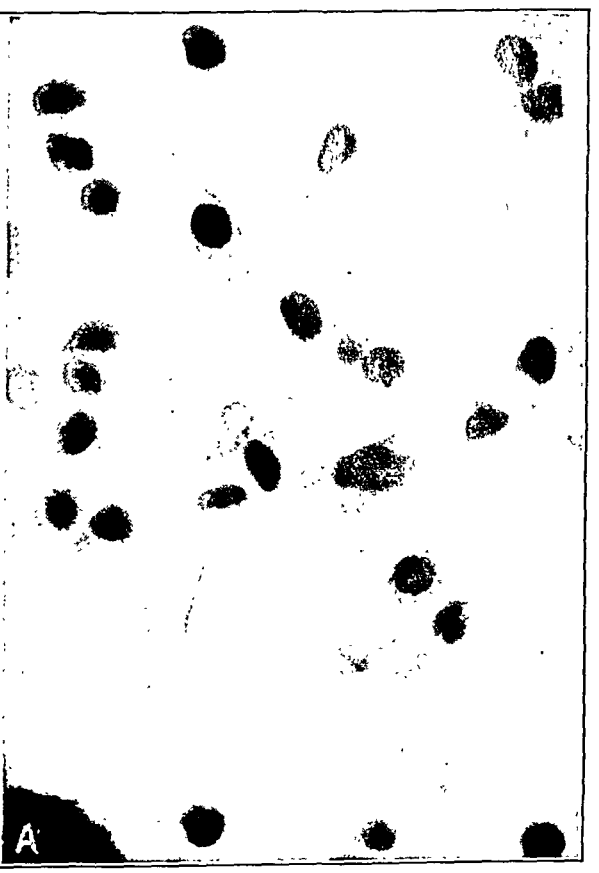
Fig. 3. Showing an area of Fig. 4 under higher magnification to illustrate the reticular pattern of growth and the grouping and connections of cells.



1



2



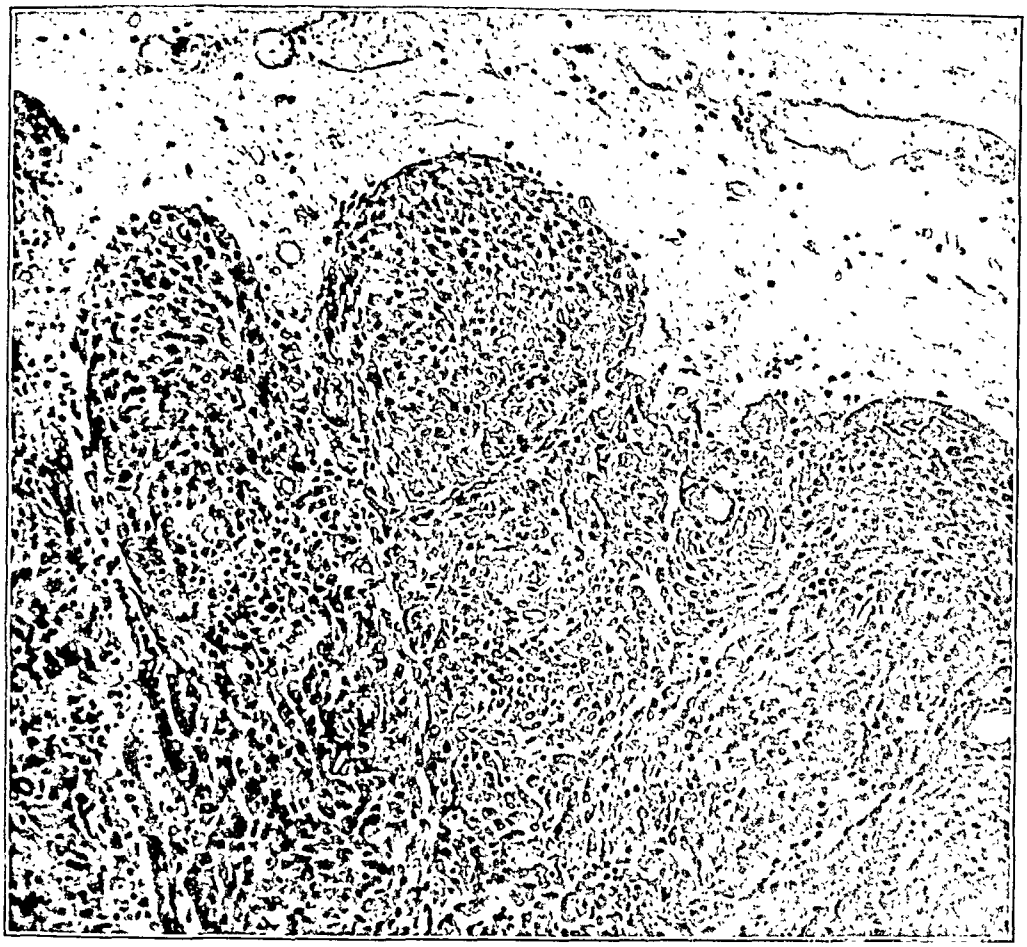
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Buckley and Eisenhardt Study of a Meningioma

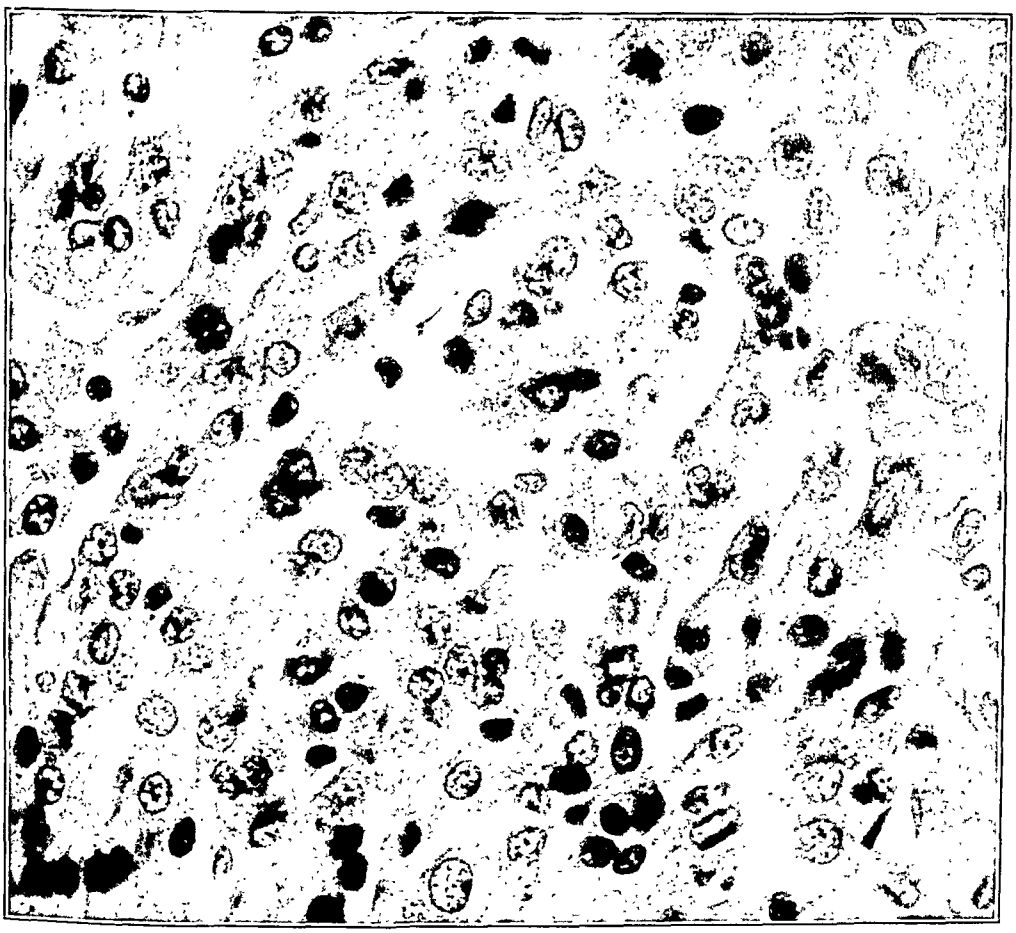
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Fig. 4. Section taken from the edge of the meningioma to show the sharp demarcation between tumor and compressed but uninvaded brain tissue. Hematoxylin and eosin. × 150.

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* Abstract of paper presented at the meeting of the American Association of Pathologists and Bacteriologists held at Chicago, Ill., March 28 and 29, 1929.

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